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THE PROSTATE GLAND OF *HERPESTES EDWARDSII EDWARDSII*

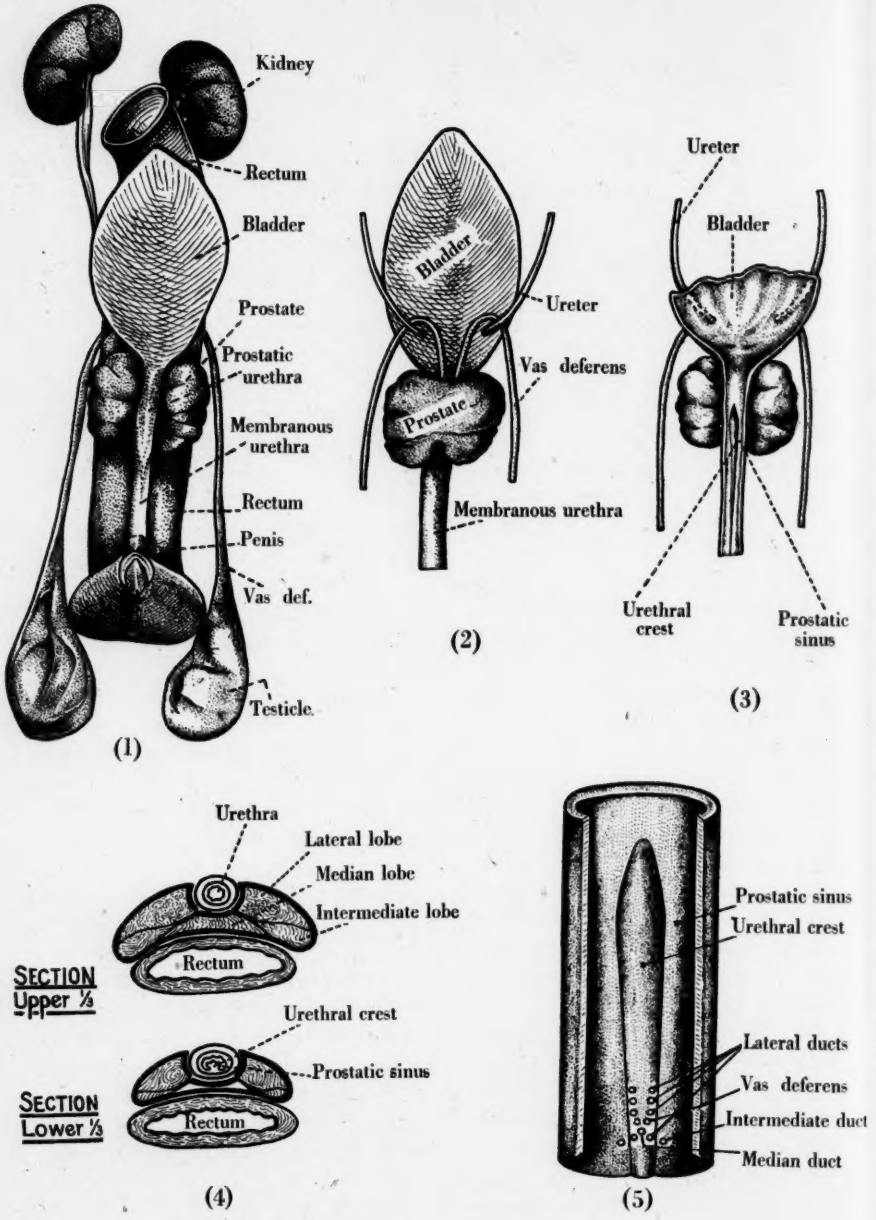
By M. A. H. SIDDIQI
King Edward Medical College, Lahore

The specimens of the mongoose, *Herpestes edwardsii edwardsii*, that I have studied were obtained from Madras. The complete animals were fixed in formalin and subsequently the neck of the bladder, prostate gland, prostatic urethra, and terminal parts of the vasa deferentia were removed *en bloc*, embedded in paraffin and cut in a complete series of transverse sections 8–10 μ. The sections used were stained with haematoxylin and eosin. Three such complete series were studied.

The prostate gland is a solid structure enclosing the urethra on the sides and dorsally (Text-figs. 1–3). It measures about $\frac{3}{4}$ in. in its anteroposterior and ventrodorsal diameters. It presents two rounded ventrolateral surfaces, a smooth concave dorsal surface, and it is grooved along its ventral side for accommodation of the urethra to which it is joined by connective tissue and the ducts of the gland.

The vas deferens passes from the testis towards the dorsal surface of the bladder, where it loops over the ureter, and then runs close to its fellow of the opposite side at first between the prostatic urethra and the gland; it then pierces the wall of the urethra obliquely, running for some distance in the submucous coat, and finally opens on the summit of the urethral crest. There is nowhere any evidence of a diverticulum of the nature of a seminal vesicle rising from the vas.

The urethra is divisible into three parts, prostatic, membranous, and penile, of which only the prostatic need concern us here. This is a straight tube about $\frac{3}{4}$ in. in length and is enclosed by the prostate gland dorsally and on the sides, its ventral surface being thus exposed to view (Text-figs. 1–3). When slit open along its ventral surface the urethral crest is seen as a median elevation in the distal half of the urethra, and it gradually tapers away when traced towards the membranous urethra. On microscopic examination the prostatic urethra shows a well-developed muscular wall, a submucous coat in which there run the vas and the prostatic ducts, and a mucous coat. The urethral crest, which is composed of mucous and submucous coats, forms a prominent elevation in the middle line so that in transverse section the urethra has a crescentic outline with a deep groove, the prostatic sinus, on each side of the crest. The openings of the vas and prostatic ducts cannot be seen by the unaided eye but can be seen with the aid of a lens. On microscopic examination



Text-figs. 1–5. Drawings of the prostate and related structures.

Fig. 1. From ventral surface. Fig. 2. From dorsal surface.
Fig. 3. From ventral surface after opening bladder and urethra.
Fig. 4. Transverse sections through the upper and lower third of the gland.
Fig. 5. Diagram to show openings of ducts.

it is found that the prostate discharges its secretion into the urethra through eleven ducts, four pairs of lateral ducts, one pair of intermediate, and a single large median duct. These ducts are associated with certain lobes of the gland which are arranged as follows:

Lobes and ducts of the prostate gland. Study of transverse serial sections shows that the gland is made up of five distinct lobes, a large median lobe, a pair of large lateral lobes, and a pair of small intermediate lobes (Text-fig. 4). Each of these five lobes has its own separate set of ducts which pierces the muscular wall of the urethra and runs obliquely for some distance in the submucous coat before opening into the urethra. The ducts open into the urethra in the following order from above downwards: lateral lobe ducts, median lobe ducts, intermediate lobe ducts (Text-fig. 5).

Lateral lobe ducts. There are about twelve of these from each lobe. They gradually pierce the wall of the urethra and run in the submucosa lateral to the vas and under cover of the mucous lining of the prostatic sinus. They join with one another until their number is reduced from twelve to four, and these then open at various levels on the sides of the urethral crest, three pairs being above the level of the opening of the vas and one pair below this.

Median lobe ducts. There are about twelve of these arranged in two main groups, and they all ultimately join to form a single median duct that opens on to the urethral crest in the middle line between the openings of the vasa deferentia.

Intermediate lobe ducts. A single collecting duct issues from each of the intermediate lobes. The duct runs down under the mucous membrane of the prostatic sinus lateral to the lateral lobe ducts and opens, one on each side, into the prostatic sinus below the opening of the vas. These are the most distal of all the duct openings.

Seminal vesicle and prostatic utricle. As has been pointed out above, the seminal vesicles are not present in *H. edwardsii*. The prostatic utricle also is absent. The median lobe and its single duct occupy the position of the utricle, and if the intramural portion of the duct only were studied and not the intraglandular portion, the duct might well be mistaken for the prostatic utricle. But on careful examination it is clear that there is nothing in the nature of the blind *cul de sac* that one usually associates with the term prostatic utricle.

Nevertheless, it should not be overlooked that the median lobe of the prostate might be regarded as being a glandular structure that has grown out from the fused distal ends of the paramesonephric ducts. In this connexion it is interesting to note that Swyer (1944) showed a photograph of a 'complex glandular type of uterus masculinus' in the prostate of a 5-year-old child. Thus though the prostatic utricle in the usually accepted sense of the term must be regarded as absent in this animal, it cannot be said that there has therefore been a complete atrophy and retrogression of the distal part of the paramesonephric duct.

SUMMARY

1. The prostate gland and prostatic portion of the urethra in *Herpestes edwardsii edwardsii* have been studied by gross dissection and microscopic examination of serial transverse sections.

2. The prostate is made up of five lobes, two lateral, two intermediate and one median.

3. The lateral lobes drain each by four ducts which open on the sides of the urethral crest. The intermediate lobes drain by two ducts which open into the prostatic sinus. The median lobe drains by a single duct which opens in the middle line on the urethral crest.

4. The seminal vesicles are absent and thus the opening of the vas is not a true common ejaculatory duct.

5. The prostatic utricle is not present as a short cul-de-sac, but its place is taken by the duct of the median lobe of the gland.

6. It is suggested that the median lobe may be derived as an outgrowth from the fused distal ends of the paramesonephric ducts.

My thanks are due to Dr T. V. Mathew, of Vizagapatam and to Mr K. I. Verghase, of Madras for collecting the specimens for me; and to Dr M. Taqi of my laboratory for help in the work.

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THE PRIMARY OPTIC CENTRES OF THE RAT. AN EXPERIMENTAL STUDY BY THE 'BOUTON' METHOD

By W. J. H. NAUTA and J. J. VAN STRAATEN
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A great number of investigations on the central termination of the optic nerve in mammals has firmly established the fact that retinal fibres end in the superior colliculus and in the dorsal nucleus of the lateral geniculate body. In addition, the subthalamic nucleus of Luys and the nucleus opticus tegmenti have been claimed to receive optic fibres in several lower animals. Lastly, physiological data (Ranson & Magoun, 1933) strongly suggest that the pretectal region contains another primary optic centre with special importance for the pupillo-constrictor reflex.

Hitherto morphological data in this field have almost exclusively been obtained by the study of normal material and by experimental studies conducted either by the Marchi technique or by the method of transneuronal cell atrophy.

In recent years the study of the end-feet of Held-Auerbach (boutons), which become unusually conspicuous after section of afferent tracts, has yielded valuable information regarding synaptic relations in the lateral geniculate body (Glees & Clark, 1941; Glees, 1941). No complete determination of their distribution in the central nervous system after section of the optic nerve has, however, been attempted so far. For this reason we publish our findings, which are based on the bouton method and which differ in some points from the results obtained by the more usual methods.

MATERIAL AND METHODS

Three albino rats were employed. Both eyes were removed from one, and the left eye only from each of the others. After survival for 5 days the animals were killed by ether and perfused through the left ventricle with saline, followed by 80 % alcohol. The brains were carefully removed and placed in 96 % alcohol for 3 days. The brain of one of the unilateral cases was then prepared by Bodian's protargol method and those of the other two according to Ranson's pyridine silver technique.

The value of the bouton method has been much discussed. Phalen & Davenport (1937) have shown that the size and shape of normal boutons in the spinal cord vary widely in different individuals and in different species. Their conclusion is that only those changes in the usual appearance of boutons which are massive in extent can be relied upon for localizatory purposes. In our experiments, however, this restriction played no important part, since we constantly failed to find more than a very few solitary boutons in six normal

rat brains stained either with the pyridine silver or with the protargol technique, whereas the optic centres in our experimental series showed a wealth of heavily staining ring- and bulb-shaped structures, in all respects comparable to those described by Hoff (1932), Glees & Clark (1941) and others (Pl. 1, fig. 2).

Meyer & Meyer (1945) have recently suggested that the appearance of heavily staining boutons in some particular region depends on physico-chemical changes in that region causing an increased argentophilia of pre-existent structures. Since they were able to demonstrate abnormally argentophil boutons in normal biopsy material, various not necessarily degenerative factors seem to be able to induce such changes. There can, however, be no doubt that at least the great majority of the boutons in our experimental material represented degenerative changes of the optic system, since after unilateral eye removal the ratio between ipsi- and contralateral boutons was found to correspond roughly with the results of previous experiments regarding the distribution of retinal fibres, in so far as more were found in the contralateral than in the ipsilateral optic centres. Although we have tried to establish this ratio by means of counting the boutons, it is doubtful whether the absolute number of boutons found in a nucleus is of value for estimating the number of fibres ending in it, since it is not yet possible to state that every degenerating fibre develops one or more degenerating boutons, and we cannot exclude the possibility that some of the boutons were not 'boutons terminaux' but bouton-like structures developing in the course of degenerating fibres ('boutons de passage', recently depicted by Meyer & Meyer, 1945).

RESULTS

Removal of both eyes gave results essentially similar to those following removal of one eye: it is the results of the latter operation that are described.

Lateral geniculate body

(*a*) *Nucleus dorsalis.* A large number of heavily staining ring- and bulb-shaped structures were found in the dorsal nucleus. The right nucleus contained roughly twice as many as the left. No definite localization of the boutons in the nucleus could be detected. On both the right and left side their number was largest in the superficial part of the nucleus and regularly decreased from without inwards, while the boutons were rather evenly distributed over the dorsoventral diameter of the nucleus. On this point our results do not correspond with those obtained in rodents and marsupials by other methods (Brouwer, Zeeman & Mulock Houwer, 1922; Jefferson, 1940; Bodian, 1937), which seems to prove that uncrossed fibres end chiefly in the dorsomedial part of the caudal half of the nucleus.

(*b*) *Ventral nucleus.* Extremely few boutons could be found in the ventral nucleus of the lateral geniculate body. Previous investigations have already indicated that the Marchi reaction in the ventral nucleus following eye removal is due to degeneration of fibres of passage and not of terminal fibres.

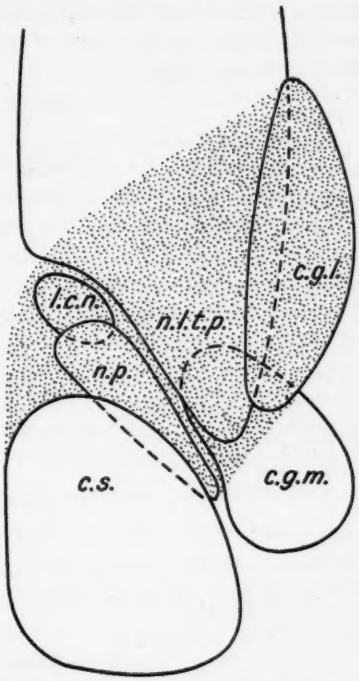
Pretectal area

After passing the lateral geniculate body and the lateral nucleus of the thalamus a considerable portion of the optic tract comes into close relation to the two main nuclei of this region, viz. (Clark's nomenclature, 1932):

(1) *The large-celled nucleus of the optic tract* (Cajal's (1911) noyau de la voie optique bigéminale), a small ovoid cell group occupying a rostromedial position in the pretectal area, just dorsal to the fibres of the commissura posterior (Text-fig. 1; Pl. 1, fig. 1).

(2) *The pretectal nucleus*, a much larger group of small cells situated immediately underneath the optic tract (Text-fig. 1; Pl. 1, fig. 1).

The rostral pole of the pretectal nucleus overlies the caudal one-third of the large-celled nucleus. The pretectal nucleus extends in a caudal and lateral direction. Its middle part lies between the rostral part of the superior colliculus medially and the nucleus lateralis thalami pars posterior (pulvinar) laterally, while its caudal part extends beyond the caudal tip of the pulvinar, and is thus situated between the colliculus medially and the medial geniculate body ventrolaterally. On the medial side the nucleus is continuous with the stratum opticum of the superior colliculus. Many optic fibres apparently pass through the pretectal nucleus only to enter the stratum opticum.



Text-fig. 1. Free-hand schematic reconstruction of the relative position of the nucleus lateralis thalami pars posterior (*n.l.t.p.*); lateral and medial geniculate bodies (*c.g.l.* and *c.g.m.*); large-celled nucleus of the optic tract (*l.c.n.*); pretectal nucleus (*n.p.*) and superior colliculus (*c.s.*), and their relation to the optic tract (shaded).

Previous anatomical studies have adduced only meagre evidence for the termination of retinal fibres in the pretectal region. In normal material Cajal (1911) observed optic collaterals entering the 'noyau de la voie optique bigéminale' (i.e. the large-celled nucleus) of the mouse, and Tsai (1925) reported similar findings for the pretectal nucleus in the opossum. The Marchi method has often yielded negative results (Bodian (1937) in the opossum; Jefferson (1940) in the ferret; Packer (1941) in the phalanger; Nichterlein & Goldby (1944) in the sheep). Clark (1931), however, observed some Marchi reaction

in the large-celled nucleus of the rat following eye removal, and Lashley (1934) reported similar findings, without, however, being certain that optic fibres ended in the nucleus. It is possible that the cellular atrophy in the thalamus opticus of the goat and cat, observed by Minkowski (1920) after eye removal, involved the pretectal area also. As a whole, the available data do not seem to offer a satisfactory morphological basis for the activity of the pretectal area in the pupillo-constrictor reflex (Ranson & Magoun, 1933; Magoun, Atlas, Hare & Ranson, 1936).

Large-celled nucleus

In our experimental material the large-celled nucleus contained such an enormous number of heavily staining boutons (Pl. 1, fig. 2 *c*) that we may safely state that no comparable concentration of these structures can be demonstrated in any of the other primary optic end-stations after eye removal. The neuropil, which apparently consists chiefly of unmyelinated collaterals of fibres of the overlying optic tract, stains much more heavily than in normal preparations. Although the ipsilateral nucleus contains a very considerable number of boutons, the changes are greatest in the contralateral nucleus. By virtue of its greater content of argentophil elements the contralateral nucleus stains more darkly than its fellow of the opposite side, a phenomenon which is already evident under low magnification (Pl. 1, fig. 1). In general, the boutons are somewhat smaller than in the other optic centres (Pl. 1, fig. 2).

Pretectal nucleus

A considerable number of boutons had appeared in the contralateral pretectal nucleus. Boutons were most numerous in the dorsal part of the nucleus. It was striking that practically no boutons could be found in the ipsilateral pretectal nucleus.

Nucleus lateralis thalami pars posterior

This part of the thalamus is bordered by the lateral geniculate body on its lateral side and by the pretectal area on its medial side; it is covered with a great number of optic fibres. The attenuated hindpart of the nucleus extends beyond the caudal pole of the lateral geniculate body to a point dorsal to the medial geniculate body, the pretectal nucleus being applied to its medial side (Text-fig. 1).

Although a large part of the thalamus is covered with optic tract fibres, degenerating boutons were found only in that portion of the lateral thalamic nucleus which lies on a level with the pretectal nucleus. Their number was considerable in the contralateral and only slightly less in the ipsilateral nucleus. Marchi granules following retinal lesion have been described by Brouwer *et al.* (1922) in the lateral thalamic nucleus of the rabbit, and Minkowski (1920) reported bilateral cell atrophy in the posterior part of the same area of the goat and cat 6 months after enucleation of one eye. It seems that recent studies with the Marchi method have failed to confirm these observations.

Superior colliculus

Our findings in this centre largely correspond with those reported by Jefferson (1940) from Clark's laboratory. There was a large number of ring and bulb formations in the crossed colliculus, especially in the stratum griseum superficiale, but to a minor extent also in the stratum opticum and stratum zonale. Very few were present in the ipsilateral colliculus, but we cannot confirm Jefferson's statement that it was entirely devoid of abnormal boutons. Those that were present were inconsistently distributed over the three superficial layers.

Nucleus opticus tegmenti

A small number of degenerating boutons was found in this cell group and a few were present in the tractus peduncularis transversus also, very near its entrance into the nucleus. There were fewer in the ipsilateral than in the contralateral nucleus.

'Noyau de la bandelette optique'

We could identify this small cell group which Cajal (1911) described in the mouse as a centre receiving collaterals from the optic tract. It is a small nucleus situated in a shallow triangular depression of the basal surface of the cerebral peduncle. Only in one section did we observe one single ring structure, and it hardly seems possible to attach any value to this solitary finding. Our preparations suggest that the nucleus is related to the ansa peduncularis rather than to the tractus opticus.

Nucleus subthalamicus (Luys)

A connexion of the retina with the subthalamic nucleus by means of the anterior accessory optic tract has been claimed for the rabbit by Bochenek (1908) and Loepp (1912). In our preparations no boutons could be found in the subthalamic nucleus of either side. This seems to confirm the results of many later observations (see Jefferson, 1940) which tended to exclude the existence of retinal fibres ending in the corpus Luysii.

Nucleus supraopticus

In the guinea-pig, Collin (1935) described unmyelinated collaterals from the chiasma and the optic tracts, which ramified and ended with end-rings or bulbs on the surface of cells of the nucleus supraopticus. Our preparations did not show any change of the intercellular fibres from their normal appearance, and no boutons could be found in the nucleus.

DISCUSSION

The most impressive feature of our experimental material was the abundance of degenerating boutons in the pretectal area, indicating a much more important relation of the retina to this area than previous anatomical findings had led

us to believe, and substantiating the physiological evidence of optic synapses in the pretectal region adduced by Ranson & Magoun (1933) and Magoun *et al.* (1936). It is of interest that while the large-celled nucleus, which contained the greatest concentration of these structures, apparently receives a considerable number of uncrossed retinal fibres, the pretectal nucleus seems to be almost exclusively related to the contralateral retina. In this respect the pretectal nucleus is comparable to the superior colliculus, which seems to favour Bodian's (1939) conception of the nucleus as a mesencephalic rather than a diencephalic structure. The large-celled nucleus, on the other hand, would seem to be more closely related to the diencephalic optic centres, since the termination of the optic nerve in the lateral geniculate body and the pulvinar is also strongly bilateral.

It was a matter of some surprise to us that we failed to find any definite localization of crossed and uncrossed boutons in the dorsal nucleus of the lateral geniculate body. It is possible that a number of degenerative ring-shaped or solid nodular swellings in the preterminal course of optic fibres masked the absence of true optic terminals in the dorsomedial part of the contralateral nucleus. It would be of interest to repeat our experiments in mammals possessing a laminated geniculate body, because more precise localization of crossed and uncrossed optic endings has proved to be possible in such forms. Although there is thus a possibility that a point-for-point relationship between related centres cannot be established by the bouton method, its value for gross localization is unchallenged, since bouton-like structures do not develop along the entire cellulo-fugal course but only in the end-stations of interrupted pathways. A striking example of this rule is furnished by the fact that the ventral nucleus of the lateral geniculate body contains practically no boutons after uni- or bilateral eye removal, although the nucleus is penetrated by a large number of optic fibres destined for the dorsal nucleus.

SUMMARY

1. Five days after removal of one eye degenerating boutons were found in the dorsal nucleus of the lateral geniculate body, the nucleus lateralis posterior thalami, the pretectal area, the superior colliculus and the nucleus opticus tegmenti.

2. Scarcely any boutons could be found in the ventral nucleus of the lateral geniculate body or in the 'noyau de la bandelette optique' (Cajal), and none at all in the corpus Luysii or in the nucleus supraopticus. In the large-celled nucleus of the optic tract (pretectal area) degenerating boutons were exceedingly numerous.

3. A considerable number of uncrossed fibres appear to end in the lateral geniculate body, the nucleus lateralis posterior thalami, and the large-celled nucleus of the optic tract, while the superior colliculus and the pretectal nucleus evidently receive very few fibres from the ipsilateral retina.

4. The material studied did not allow of any definite localization of crossed and uncrossed optic terminations in the dorsal nucleus of the lateral geniculate body. Areas receiving either crossed or uncrossed optic fibres have been demonstrated in the nucleus of rodents with other methods.

5. It remains for further study to decide whether the bouton method is of value for establishing point-for-point relationships between centres.

The authors wish to thank Mr O. Kerssen for the drawing and the photographs which illustrate this paper.

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EXPLANATION OF PLATE

Fig. 1. Part of the pretectal area, 5 days after unilateral eye enucleation. The large-celled nucleus (*l.c.n.*) has stained darker on the crossed (left half of the figure) than on the uncrossed side. Bodian's technique. ×50.

Fig. 2. Degenerating boutons in several of the contralateral optic centres, 5 days after enucleation of one eye. *a*, dorsal nucleus of the lateral geniculate body; *b*, superior colliculus; *c*, large-celled nucleus of the optic tract; all three taken at same magnification (×1100). Pyridine-silver technique.

Key to lettering

c.p. commissura posterior.
l.c.n. large-celled nucleus of the optic tract.
n.p. pretectal nucleus.

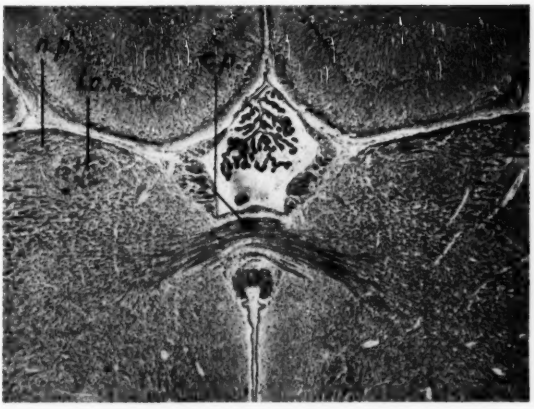
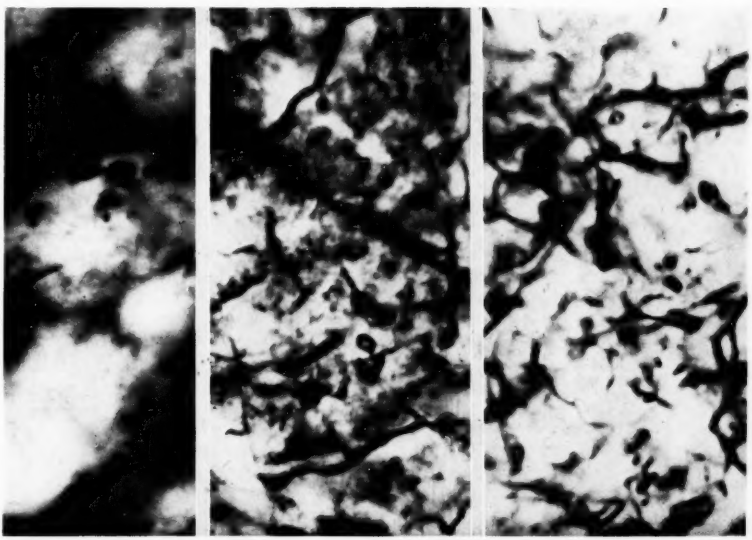


Fig. 1.



a *b* *c*

Fig. 2.

ABSENCE OF CELL MULTIPLICATION DURING DEGENERATION OF NON-MYELINATED NERVES

By J. JOSEPH, *Department of Anatomy, University College, London*

INTRODUCTION

One of the most remarkable features of the degeneration which follows severance of a myelinated nerve is that the multiplication of the nuclei of Schwann occurs not only in the neighbourhood of the lesion but throughout the whole extent of the peripheral stump. Abercrombie & Johnson (1946) have recently examined this multiplication quantitively in the sciatic nerve of the rabbit and have found that the increase occurs between the 4th and 25th days and produces a Schwann cell population thirteen times that of the normal nerve. Information about the agency which produces this nuclear multiplication might provide us with valuable clues as to the nature of cell proliferation.

Few previous studies have dealt with the degeneration of non-myelinated nerve. Tuckett (1896) observed that when non-myelinated fibres are cut off from their nerve cell and degeneration is shown by loss of irritability, 'the nuclei and walls of the fibres seem to be quite unchanged and as normal as ever'. Other writers had different impressions. Ranson (1912) stated that 'by the 19th day after degeneration of non-myelinated nerve the nuclei of the neurilemma have greatly increased in number', and Cajal (1928) described a thickening and proliferation of the cells surrounding the non-myelinated nerve-fibres. The only other reference found to histological changes was the work of Machida (1929) who showed a picture of a longitudinal section showing 'proliferation of the neurilemma', but who stated in the text that 'the neurilemma was essentially unchanged'. Tuckett's excellent paper is open to the criticism that it is now known that the nerve he used contained a large number of finely myelinated nerve-fibres, an error due to the stage of technical advance reached at that time.

The present paper describes an attempt to determine the quantitive changes in the cell population of a non-myelinated nerve which has been interrupted.

MATERIAL AND METHOD

The nerve used was the anterior mesenteric of the rabbit, which has been shown by Simpson & Young (1946) to consist of a number of bundles containing only a few (total less than ten) small myelinated fibres. The nerve was crushed with smooth-tipped forceps, or cut, just distal to the anterior mesenteric ganglion. After 21 days the ganglion and nerve were removed. These constituted two specimens each about 1 cm. long, one consisting of the ganglion and proximal part of the nerve and the other of the distal part of the nerve. The specimens were fixed in Bodian's fluid containing 15 c.c. formaldehyde, 5 c.c. acetic acid

and 80 c.c. of 80% alcohol, and were embedded in paraffin. Transverse sections at 5 μ of each end of each specimen were cut, and four slides of each end were prepared. One slide was stained for axons by Bodian's method, one by Bodian and Mallory's stain, one by Bodian and Masson's stain and one with haematoxylin and eosin. An equal number of normal ganglia and nerves were prepared in a similar way. Longitudinal sections of both the interrupted and normal nerves were cut at 7 μ and used in the case of the interrupted nerves chiefly to determine the existence of normal axons proximal to the interruption and their absence beyond. Sections showing at least three satisfactory nerve bundles stained with haematoxylin and eosin, in each specimen, were used for counting. Photographs of these sections were taken at a magnification of $\times 350$, and the number of nuclei in each of the three nerve bundles was counted. No attempt was made to define the type of cell to which the nuclei belonged. The area of each nerve bundle was measured with a planimeter, the outline followed being that of the perineurium from which the nerve bundle had invariably shrunk away to a greater or less extent. The number of nuclei in each nerve bundle was expressed as so many per 10,000 μ^2.

RESULTS

Table 1 shows the counts for three nerve bundles in each of five normal nerves, and Table 2 shows those for a similar number of bundles in nerves which had been interrupted.

Table 1

Serial no. of rabbit	No. of nerve bundle	Nuclear count	Area in 10,000 μ^2	Nuclei per 10,000 μ^2
49	1	40	0·56	72·06
	2	24	0·33	72·14
	3	68	0·91	74·55
47	1	81	1·46	56·04
	2	24	0·31	81·14
	3	24	0·30	80·00
72	1	164	2·81	58·34
	2	38	0·74	51·53
	3	143	2·00	71·50
48	1	26	0·38	68·24
	2	144	2·59	55·53
	3	46	0·52	88·51
110	1	46	0·51	89·91
	2	94	0·75	125·17
	3	72	0·87	82·43

Mean = 74·95.
Standard deviation = $\pm$ 18·24.

The nerve in rabbits 13, 19, 12 and 23 was interrupted by crushing and that in rabbit 27 by cutting.

The Bodian staining showed that a number of axons had regenerated but these were very fine at the levels studied. This should not affect the results but in confirmation a further experiment is being performed in which regeneration is completely prevented.

The estimates of the number of nuclei per 10,000 μ^2 both in the normal and damaged nerves vary considerably in different animals. These differences can be accounted for by factors such as slight obliquity of the sections, and variation in the degree of shrinkage of the nerve bundles, besides some normal variation in the size of the nuclei and their density along the length of the nerve. On the whole, variation within the limits shown in the Tables is to be expected in counts of this type.

Comparison of the means and standard deviations of the two sets of results allows us, in spite of the variation, to assert that the nuclear population of a non-myelinated nerve 21 days after it has been interrupted is not greater, by a factor of two, than in the normal nerve. Probably there has been no cell multiplication at all, certainly it has not affected every cell in the nerve. This is in very striking contrast to the multiplication by eight times which occurs during degeneration of myelinated nerves.

Table 2

Serial no. of rabbit	No. of nerve bundles	Nuclear count	Area in 10,000 μ^2	Nuclei per 10,000 μ^2
13	1	95	1·38	68·81
	2	124	1·80	69·04
	3	228	4·36	52·30
19	1	119	2·26	52·68
	2	90	1·35	66·48
	3	8	0·10	80·00
12	1	226	2·04	110·74
	2	45	0·73	61·25
	3	96	1·16	83·00
23	1	60	0·91	66·02
	2	51	0·81	62·68
	3	41	0·79	52·14
27	1	128	1·33	96·00
	2	41	0·75	54·99
	3	66	1·59	41·39

Mean = 75·56.
Standard deviation = ± 18·12.

DISCUSSION

The exact histological details of the structure of mammalian non-myelinated nerve have not yet been decided. One of the main problems is whether or not there is a complete absence of myelin between the neurilemma and the axon. The fullest description that I have found is that given by Nageotte (1932). He describes an axon surrounded by a 'sheath of Schwann' which has a 'membrane of Schwann' outside it, and states that there is no myelin sheath. He considers that the sheath is syncytial and is irregularly studded with elongated nuclei and that the membrane in cross-section forms a multitubular structure with an axon in each tube. The cells of this sheath (cells of Remak) are usually regarded as Schwann cells. Ranson (1911) described what he called a 'halo' round the axons of non-myelinated nerve bundles in spinal nerves, and

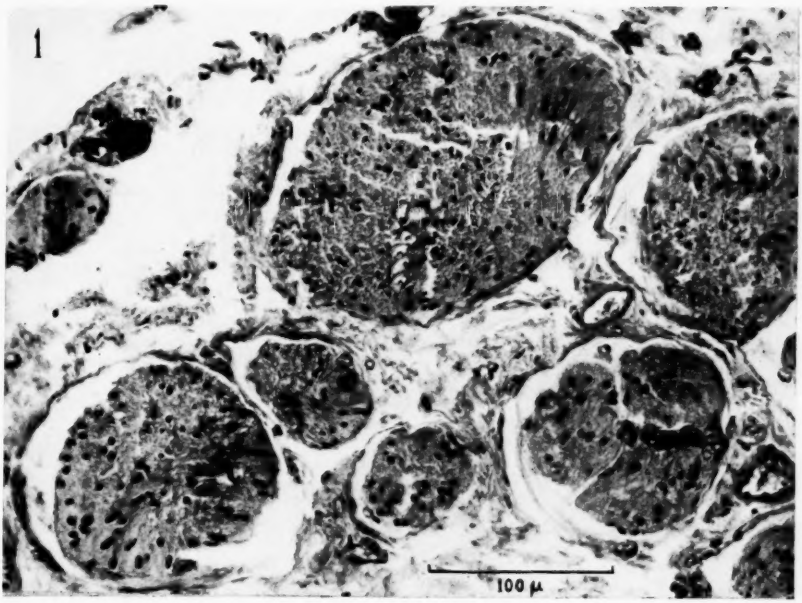
suggested that the 'halo' may be a definite sheath apart from the neurilemma. This sheath may be a layer of fatty molecules around the axon, i.e. a myelin sheath which contains too little fat to be demonstrable after treatment with osmium tetroxide (Schmitt & Bear, 1939). Ranson, however, could find no trace of nodes of Ranvier. The terminology is confusing but comparison with the structure of a myelinated nerve will help to clarify the position. In the myelinated nerve it is important to distinguish between the Schwann cell outside the myelin sheath and the neurilemma outside the Schwann cell. On the other hand, in the non-myelinated nerve both these layers are called the neurilemma ('sheath of Schwann' of Nageotte) and the outer layer the endoneurium ('membrane of Schwann' of Nageotte). Nageotte's description of the 'membrane of Schwann' correponds to what Gaskell (1886) and Ranson (1911) both call the background of 'connective tissue' in which non-myelinated axons appear. The nuclei within the perineurium bounding a non-myelinated nerve bundle are either those belonging to the neurilemma (these may or may not be equivalent to the Schwann cell nuclei of a myelinated nerve) or connective tissue nuclei or blood-vessel nuclei. No differentiation between these nuclei was attempted in the present work.

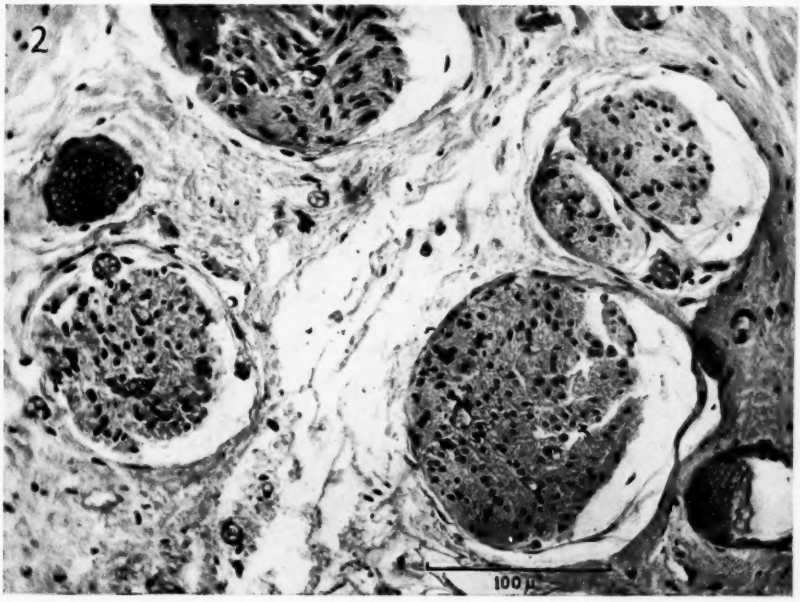
Abercrombie & Johnson (1946) have studied the changes in nuclear density in the degenerated sciatic nerve of a rabbit at varying intervals after interruption of the nerve. They point out that the maximum increase is after 25 days, the tubal nuclei, i.e. Schwann cell nuclei, having then increased to thirteen times and the endoneural nuclei, i.e. all the others, to four times their original number, the overall increase being 8·4 times. The difference between their results and those in this paper is very striking. There are several possible explanations for this difference. The absence of myelin leads to two obvious differences between the process of degeneration in non-myelinated and myelinated nerves. In the first place the space left within the sheath is small when the small non-myelinated fibres degenerate. Secondly, there are no myelin remains to provide a chemical stimulus to division of the cells. The failure of the cells of degenerating non-myelinated nerve to divide suggests that one of these two factors is responsible for causing the multiplication in myelinated nerves. It must not be forgotten, however, that the nature and origin of the nuclei in both cases is still somewhat uncertain and we cannot be sure that they are strictly comparable. Denny-Brown (1946) has recently claimed that many of the cells normally regarded as Schwann cells are a special type of 'neural fibroblast'. Further work on the nature of the nuclei in all types of nerve is needed, but for the present we may consider the cells in non-myelinated nerve essentially similar to Schwann cells.

SUMMARY

1. The anterior mesenteric nerve of the rabbit, consisting almost wholly of non-myelinated nerve-fibres, was found to show $75{\cdot}56 \pm 18{\cdot}12$ nuclei per $10{,}000\,\mu^2$ in transverse sections $5\,\mu$ thick.

2. Twenty-one days after the fibres of this nerve had been interrupted by





JOSEPH—CELL MULTIPLICATION IN DEGENERATED NERVES

crushing or severance the nuclear population in the distal stump was 74·95 ± 18·24.

3. Evidently the cell population has changed little, if at all, in contrast to the multiplication by more than eight times which occurs in the nuclei of myelinated nerve during degeneration.

4. The differences between the two types of nerve are (*a*) the lack of myelin, and (*b*) the smaller space within the neurilemma of the non-myelinated nerve. The cell multiplication in a myelinated nerve must therefore be due either to the effect of the break-down products of myelin or to the physical collapse of the large axons.

I wish to thank Professor J. Z. Young for many helpful suggestions and for reading and criticizing the manuscript; Mr J. Armstrong for technical assistance; and Mr E. G. Reeve, of the Social Survey, for advice on statistics.

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EXPLANATION OF PLATE

Fig. 1. Transverse section of normal anterior mesenteric nerve of rabbit. Sections 5 μ thick. Stained haematoxylin and eosin. (Rabbit 47.)

Fig. 2. Transverse section of anterior mesenteric nerve of rabbit distal to crush performed 21 days previously. Sections 5 μ thick. Stained haematoxylin and eosin. (Rabbit 12.)

ORGANIZATION OF THE DORSAL COLUMNS OF THE SPINAL CORD AND THEIR NUCLEI IN THE SPIDER MONKEY*

By HSIANG-TUNG CHANG and THEODORE C. RUCH

Laboratory of Physiology, Yale University School of Medicine, New Haven, Connecticut, and the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington

INTRODUCTION

The dorsal funiculi of the spinal cord are the chief pathways for the conduction of impulses underlying deep sensibility and tactile discrimination which reach the thalamus and ultimately the cortex after a relay at a bulbar level, the nucleus gracilis and nucleus cuneatus. There is a steady increase in size of the dorsal funiculi and their bulbar nuclei throughout the phylogenetic scale from lower animals to man. Amphibians and reptiles have no dorsal column nuclei and medial lemniscus. The dorsal column of these animals is small and is apparently made up of short intersegmental fibres and is different in nature from the fasciculus gracilis and fasciculus cuneatus of mammals, since there is no increase in size of the dorsal column at the cervical levels as compared with the sacral. The dorsal-column system of birds is also of a primitive type presumably due in part to the lack of discriminative sensibility of skin covered with feathers. Sea mammals, whose mode of life places no premium on a detailed knowledge of the surrounding objects and who lack prehensile and manipulative ability, have a poorly developed dorsal-column system. It is believed that the development of the dorsal funiculi and their nuclei in the mammalian and primate series is correlated with increasing sensory discrimination in the skin and the increased development of proprioceptive sense in the limb musculature.

According to Bischoff (1899), tailed animals often possess an accessory cell group situated in the median plane between the nucleus gracilis of each side. The spider monkey possesses an exceedingly well-developed tail, not only as to size and voluntary movement, but also as to proprioceptive sensibility.† It

* This paper constitutes one of the seven chapters of a dissertation, 'Segmentation, lamination and topographical projection in the nervous system with special reference to the tail of *Ateles*', presented by H. T. C. to the Faculty of the Graduate School of Yale University in candidacy for the Degree of Doctor of Philosophy. The experiments were aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

† 'In watching the performance of the spider monkey, one is profoundly impressed by the exquisite proprioceptive sense which guides its caudal movements. The animal will raise its tail to a familiar perch behind it and curl the tip about it accurately without altering its forward gaze. If the posterior perch is moved the tail comes up to the place where the perch has been, but soon discovers the change and rapidly and delicately executes the necessary adjustment' (Fulton & Dusser de Barenne, 1933).

is to be expected that the tail of the spider monkey would be strongly represented in the dorsal-column system and that a large nucleus of Bischoff would be found, but according to Kappers, Huber & Crosby (1936), a separate nucleus of Bischoff is not present in *Ateles*. This paradox suggests that knowledge of the termination of the caudal contribution to the dorsal-column system is insufficient.

The present paper describes the pattern of the cellular arrangement of the dorsal-column nuclei, and degeneration experiments on the afferent projection to these nuclei. New evidence of the specific morphological differentiation and of the functional significance of the nucleus gracilis is presented.

MATERIAL AND METHODS

The study of the cytoarchitectonics of the dorsal-column nuclei was based on serial sections of the medulla oblongata of a normal spider monkey, cut transversely at 10 μ and stained by the Nissl technique. Figures showing the subdivisions of the cell masses of these nuclei were made by tracing the images of the sections projected from an Edinger apparatus. Each cell was faithfully marked with a minute dot at its proper position as accurately as possible.

To determine the relation of different cell groups in the nuclei to the different segments of the cord, Marchi material was used. Four spider monkeys were operated upon. Two of them (SMS 13, 17) were subjected to total spinal transection at the first caudal segment; one (SMS 14) to spinal hemisection at Ca_1 and another (SMS 19) to a total transection at Ca_1 and a hemisection at L_1 with the second operation 6 months later than the first. The preparations were made according to the Swank & Davenport (1935) method.

RESULTS

Cytoarchitectonics of the dorsal-column nuclei

The dorsal-column nuclei in the spider monkey, as in most other mammals, can be divided into three main groups: (1) nucleus gracilis, (2) nucleus cuneatus, and (3) the external cuneate nucleus.

(1) Within the nucleus gracilis of the spider monkey there can be distinguished two subgroups of cells, the pars dorsomedialis and pars ventrolateralis. They stand as two distinct groups of cells throughout their length in the lower medulla. Both parts extend as far caudal as the first cervical segment, the dorsomedial part extending more caudally still. Their position when traced from the lower to the upper levels of the medulla can be easily seen in Text-fig. 1 A–C.

Cells of both parts of the nucleus gracilis are cytologically similar. They are small and medium-sized, triangular, stellate or oval cells with comparatively scanty cytoplasm.

There is no distinct cell mass lying in the midline which can be identified with the median nucleus of Bischoff, although not infrequently a few cells of the two sides tend to approach each other in the lower half of the nucleus.

(2) The nucleus cuneatus (Text-fig. 1) begins and ends higher than the nucleus gracilis. The cells in this nucleus are larger and more densely and uniformly distributed. There is little indication of lamination.

The cell volume of the nucleus cuneatus in the spider monkey is no more than one-half that of the nucleus gracilis (two parts combined). The relative size of the nucleus cuneatus in the spider monkey is apparently much smaller than in man, in whom it is as large as, or even larger than, the nucleus gracilis.

The subdivision of the nucleus cuneatus into a pars triangularis and a pars rotunda, as suggested by Ferraro & Barrera (1935), in the macaque is not always discernible in the spider monkey. Another difference is that the region labelled by them nucleus gracilis corresponds in our sections to the sensory nucleus of the vagus.

Between the nucleus cuneatus and the pars ventrolateralis of the nucleus gracilis, there is a wedge-shaped layer of cells, which is topographically more closely related to the nucleus gracilis, but is more similar in cell structure to the nucleus cuneatus. This layer of cells, as we shall see later, receives terminations of the dorsal column fibres arising from the thoracic segments.

(3) The external cuneate nucleus (Text-fig. 1 C) is situated at the dorsolateral corner of the medulla and is characterized by the presence of large, round and polygonal cells rich with cytoplasm. It is easily recognized in the medulla.

Distribution of degenerated fibres in the dorsal column and its nuclei after caudal lesions

The long-ascending fibres in the dorsal column originating from the caudal segments of the cord in the spider monkey are grouped as an independent bundle distinct from that containing the ascending bundles of lumbo-sacral dorsal root fibres. The dorsal surface of the cord is indented by a dorsal paramedian sulcus which is distinct in the lumbar region but disappears in the thoracic. In the cross-section of the lumbar cord an intermediate septum can be seen leading ventromedially from the dorsal paramedian sulcus to the middle of the dorsal median septum.

In Marchi preparations after spinal cord transection at Ca_1 (SMS 13, 17), degeneration in the lower lumbar segments is sharply confined to the triangle formed by the dorsal paramedian and dorsal median septa and to a long, thin extension from the ventral angle along the median septum to the ventral margin of dorsal column (Text-figs. 2, 3). In the central zone of this caudal bundle near the median septum there is an area containing a considerable number of undegenerated fibres. This zone may correspond to the descending fibres in Flechsig's oval area.

In the lower thoracic region (D_8) the degenerated caudal bundle is displaced to the dorsal surface of the dorsal column (Text-fig. 2, D_{10}; and Text-fig. 3, D_8), and higher up (D_4) it extends laterally to the point of entrance of the dorsal roots. The caudal fibres thus form a cap for the dorsal columns. The area occupied by the degenerated fibres corresponds topographically to Flechsig's

Fig. 1A. Fig. 1B. Fig. 1C.

Text-fig. 1. Drawings from a series of sections taken at the lower medulla showing the dorsal-column nuclei of spider monkey. *ngd*, nucleus gracilis dorsomedialis; *ngv*, nucleus ventro-lateralis; *nc*, nucleus cuneatus; *nce*, nucleus cuneatus externus.

'dorsal-root zone' of the dorsal columns. In the cervical region the caudal bundle is again back to the triangular area in the dorsomedial angle of the dorsal column. Here a septum separates the fasciculus gracilis from the

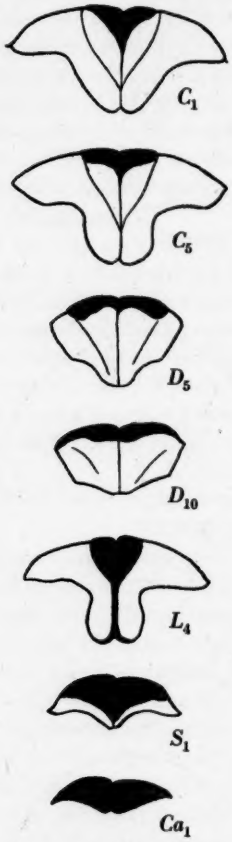

Fig. 2.

Fig. 3.

Text-fig. 2. Secondary degeneration in the dorsal column after spinal transection at Ca_1 (SMS 13).
Text-fig. 3. Secondary degeneration in the dorsal column after spinal transection at Ca_1 (SMS 17).

fasciculus cuneatus, but the septum between the caudal and lumbo-sacral bundles is no longer seen, though the demarcation is still fairly sharp.

The dorsal and lateral displacement of the caudal bundle along the dorsal surface in the thoracic region seems inconsistent with the law of lamination of long-ascending tracts whereby fibres from successive dorsal roots are arranged

in a regular order with the fibres of lowest origin at the most dorsomedial corner. Since this variation in position occurs in the thoracic region, it is possibly conditioned by the smallness of the dorsal root contributions in the

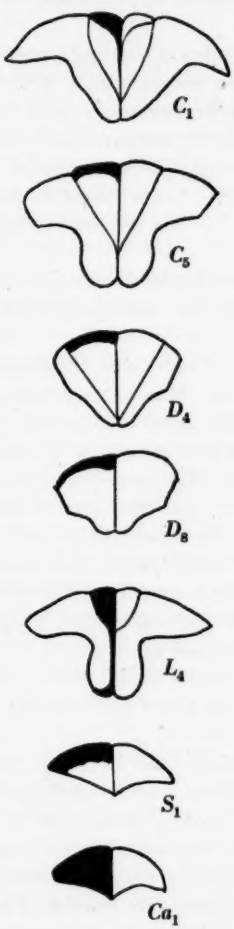

Fig. 4.

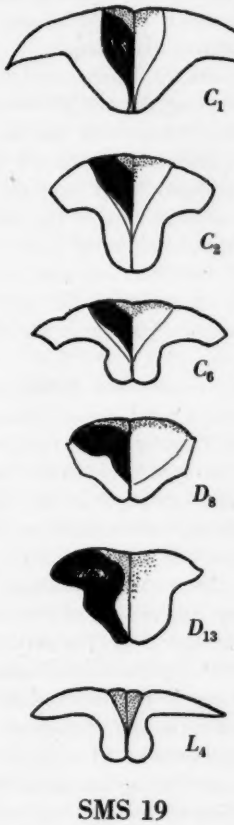

Fig. 5.

Text-fig. 4. Secondary degeneration in the dorsal column after left hemisection of the spinal cord at Ca_1 (SMS 14).

Text-fig. 5. Secondary degeneration in the dorsal column following left hemisection at L_1 6 months after a total transection at Ca_1. The stippling represents the distribution of residual degeneration products resulting from the first lesion (SMS 19).

thoracic region available to push the caudal bundle towards the midline; it therefore migrates laterally. Furthermore, in the lower thoracic region many posterior column fibres, from the lumbo-sacral roots, are terminating in Clarke's column which might occasion a re-arrangement of the regional components. However, the mechanics of the variation in position of the caudal fasciculus remains unexplained.

In the medulla the degenerated fibres are grouped in numerous small bundles interwoven among the cell clusters of the pars dorsomedialis of the nucleus gracilis. Above the level of the obex, they are further fragmented into many dark patches alternating with cell masses along the dorsomedial border of the medulla. The degenerated products become progressively smaller and are lost in the pars dorsomedialis of the nucleus gracilis. No degenerated fibres have been seen terminating in the pars ventrolateralis. Nor are they present in any other part of the dorsal-column nuclei.

Exp. SMS 14, in which the spinal cord was hemisected at Ca_1, gave the same degeneration picture in the dorsal column on the operated side (Text-fig. 4; Pl. 1, fig. 1).

In another experiment (SMS 19) the spinal cord was hemisected at L_1 6 months after a total spinal transection at Ca_1. Marchi preparations of this animal show (Text-fig. 5) the limits of the lumbo-sacral bundle. In the lumbar segments of the cord between the two lesions, the degeneration products in the caudal bundle resulting from the first operation have largely disappeared. The scattered splinters of osmium granules in it are probably partly the residual products from the first operation and partly those produced by descending fibres from the second operation. Sections taken above the second lesion present a negative picture of the caudal bundle as demarcated in SMS 13 and SMS 14 (Text-figs. 2, 4). The area occupied by fibres of caudal origin appears clearer than other parts of the fasciculus gracilis, and the degeneration products found there appear old and to date from the caudal lesion. The sparing of the area occupied by caudal fibres is clearly manifested in a photomicrograph from the upper segments of the cord (Pl. 1, fig. 2).

The most interesting feature in this experiment (SMS 19) is the distribution of the degenerated nerve terminals in the dorsal-column nuclei. Consistent with expectation, degeneration products are mainly distributed in the pars ventrolateralis of the nucleus gracilis. The pars dorsomedialis, which receives the terminations of the caudal fibres, is virtually free of degeneration products (Pl. 1, fig. 3), and the nucleus cuneatus is absolutely devoid of secondary degeneration materials. At the upper level of the pyramidal decussation, layers of degenerated fibres lie between the two parts of the nucleus gracilis so as to enhance the separation of those two cell masses. Towards the cranial end of the nucleus the degenerated fibres are broken up into fine black particles which spread exclusively throughout the pars ventrolateralis of the nucleus gracilis. The characteristic dust-like appearance round degenerated nerve endings cannot be seen in the dorsomedial part, nor can it be found in the small

layer of grey matter inserted between the nucleus gracilis and nucleus cuneatus. The degeneration picture as observed under moderately high magnification is shown in Pl. 1, fig. 3, in which the absence of fine degeneration products in the pars dorsomedialis and in the inserted cell layer between nucleus gracilis and cuneatus is clearly seen.

DISCUSSION

From observations on normal and on experimentally degenerated material, it is evident that the dorsal column of the spider monkey can be divided into three distinct fasciculi, each terminating in a distinct nuclear mass in the medulla oblongata. The dorsomedial bundle in the dorsal funiculus is derived from the caudal region and has not been previously described, although the laminated arrangement in the dorsal column of dorsal root fibres from successive body segments is well known. Kappers *et al.* (1936) correlate the separation of the dorsal column into fasciculus gracilis and fasciculus cuneatus with an increased independence in function of fore- and hindlimbs in most mammals. This suggestion, if correct, may also explain the presence of a third and separate bundle in the dorsal column of an animal with a 'fifth hand'—the prehensile tail.

The myelencephalic representation of the tail was first considered by Bischoff (1899), who, from a survey of the structure of dorsal-column nuclei of various mammals, concluded that a midline nuclear mass lying between the nucleus gracilis of each side was particularly concerned with the tail. Unpaired nuclei are seldom found in the brains of higher animals. Bischoff ascribed the exception to this rule to the axial character of the tail.

It is true that the nucleus of Bischoff occurs in many tailed animals such as rats, shrewmice, kangaroo, anteater and macaque, but not all animals having a nucleus of Bischoff possess a well-developed tail. The hedgehog is an example. According to the present study and also Ziehen (1899) and Kappers *et al.* (1936), this nucleus is not present in the spider monkey despite its highly developed tail. In the spider monkey, the cell mass which relays proprioceptive impulses from the tail is evidently the pars dorsomedialis of the nucleus gracilis which at its cephalic end is divided from its fellow by the fourth ventricle and at its caudal end is not fused with the corresponding area of the opposite side. It is therefore unlike the single midline nucleus of Bischoff. We have found a similar arrangement of the dorsal-column nuclei, especially of the nucleus gracilis, in the pangolin, which has a powerful tail used for grasping and balancing while searching in trees for nests of ants.

Besides the presence of a separate nuclear mass in the medulla for the relay of proprioceptive impulses from the tail, the dorsal-column system of the spider monkey is notable in another respect, the relative size of the nucleus gracilis and nucleus cuneatus. The nucleus cuneatus which is slightly the larger in man is undoubtedly the smaller in the spider monkey. This is probably a reflexion of the imperfect development of the hand of *Ateles*, as evidenced by

the rudimentary thumb. The hand of the spider monkey is adapted for grasping, but is incapable of the exquisite manipulative ability seen in the pentadactyl primate hand.

The position of the thoracic fibres in the dorsal column has been a subject of dispute. Some early workers (Schiefferdecker, 1877; Singer, 1882) believed that the fasciculus gracilis is composed only of the long ascending fibres from the sacral and lumbar dorsal roots. Others (Schultze, 1883; Hofrichter, 1883) believed that the dorsal roots of the thoracic nerves also contribute to the formation of the fasciculus gracilis. Recently, Foerster (1936) confirmed the latter view in man. He found that the dorsal roots of the lower six thoracic nerves join the fasciculus gracilis, while the upper six thoracic nerves contribute to the fasciculus cuneatus. The same arrangement has been described in macaques by Ferraro & Barrera (1935). Our findings in the spider monkey also agree with Foerster's result. In SMS 19, with a lesion at L_1, a narrow strip of fibres in the ventrolateral border of the fasciculus gracilis clearly remains undegenerated, and a layer of grey matter at the extreme ventrolateral border of the nucleus gracilis is also free of degeneration.

SUMMARY

The cytoarchitectonics of the dorsal-column nuclei with special reference to the subdivisions of the nucleus gracilis in the spider monkey are described from normal Nissl preparations. Two subdivisions are distinguished, pars dorsomedialis and pars ventrolateralis.

An isolated caudal bundle in the dorsomedial part of the dorsal column is demonstrable by the Swank-Davenport method in spider monkeys following transection or hemisection of the spinal cord at the first caudal segment. The fibres of this caudal bundle terminate in the pars dorsomedialis of the nucleus gracilis which has developed in the spider monkey into a large and well-formed cell group distinct from the other nuclear masses of the dorsal-column nuclei. Fibres from the lumbo-sacral segments terminate in the pars ventrolateralis. The absence of Bischoff's 'tail nucleus' in *Ateles* is confirmed.

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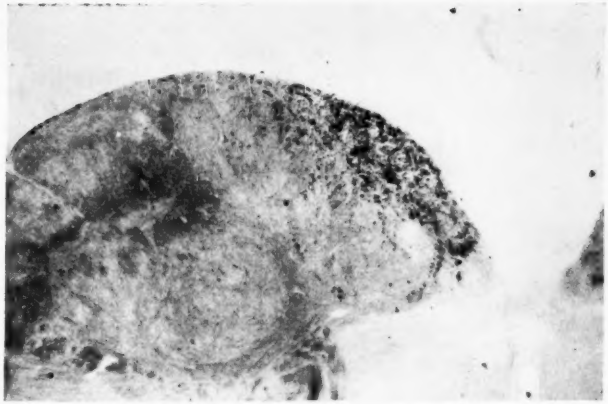

Fig. 1.

Fig. 2.

Fig. 3.

CHANG AND RUCH—DORSAL COLUMNS AND THEIR NUCLEI IN THE SPIDER MONKEY

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EXPLANATION OF PLATE

Fig. 1. Unretouched photomicrograph of the dorsal-column nuclei showing the degeneration confined to the pars dorsomedialis of nucleus gracilis after left hemisection at Ca_1 (SMS 14). Magnification ×33.

Fig. 2. Unretouched photomicrograph of a transverse section of the spinal cord at C_1 after left hemisection at L_1 6 months after a total transection at Ca_1 (SMS 19). Magnification ×18.

Fig. 3. Unretouched photomicrograph of a cross-section of medulla oblongata showing degeneration in the pars ventrolateralis of nucleus gracilis after left hemisection at L_1 6 months after a total transection at Ca_1 (SMS 19). Magnification ×24. Note the relatively slight evidence of degeneration products in the pars dorsomedialis of the nucleus gracilis and their total absence in the lamina lying between the nucleus gracilis and the nucleus cuneatus.

TOPOGRAPHICAL DISTRIBUTION OF SPINOTHALAMIC FIBRES IN THE THALAMUS OF THE SPIDER MONKEY*

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INTRODUCTION

The ventral nucleus of the thalamus receives two systems of afferent ascending fibres of ultimate spinal origin: the medial lemniscus fibres arising from the nuclei gracilis et cuneatus, and the spinothalamic fibres originating from various levels of the spinal cord. In comparison with the studies on the arrangement of the former system the topographical analysis of the thalamopetal fibres from the spinal cord is incomplete.

Many dorsal root fibres, after ascending in the dorsal column, effect synapses in the bulbar nuclei with secondary neurons which pass to the thalamus. This indirect relation of dorsal root and thalamus was discovered in the same year (1885) by Edinger and by Flechsig. The direct connexion of the spinal cord and thalamus was unknown until 1889, when Edinger observed in fish, frogs and cat embryos that cells in the dorsal horn give rise to axons which pass through the ventral commissure, ascend in the opposite anterolateral funiculus and seemingly end in the thalamus. This was soon confirmed by Auerbach (1890) in degeneration preparations. Wallenberg (1899) and Kohnstamm (1900) demonstrated the existence of such fibres in rabbits, and Tooth (1892) and Mott (1895) in monkeys. Tooth made a lesion in the lateral region of the spinal cord at the level of the first cervical nerve in the bonnet monkey and traced the degeneration as high as the pons. After division of the anterolateral fasciculus on one side in monkeys, Mott could follow a few degenerated fibres apparently going to the thalamus; but he did not make a detailed analysis of their destination within the thalamus.

In Le Gros Clark's (1936) work on the topographical arrangement of the thalamic terminations of ascending tracts in macaques only two of eight monkeys were devoted to a study of the spinothalamic tract. These were subjected to spinal hemisection at C_4–C_5 in the one case and at Th_3–Th_4 in the other. Unfortunately, the termination in the thalamus of degenerated

* This paper constitutes one of the seven chapters of a dissertation, 'Segmentation, lamination and topographical projection in the nervous system with special reference to the tail of *Ateles*', presented by H. T. C. to the Faculty of the Graduate School of Yale University in candidacy for the Degree of Doctor of Philosophy. The experiments were aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

fibres from the cervical hemisection was indeterminable owing to the presence of pseudo-Marchi granules, so that differential topographical projection could not be established.

In 1938 Walker extended this study to a chimpanzee (1938*b*) and two macaque monkeys (1938*a*). All the lesions in his experiments were at a cervical level, and he did not study the regional thalamic termination of spinal fibres from different levels of the cord. Weaver & Walker (1941) in studying the topographical arrangement of the spinothalamic tract in the brain stem carried out anterolateral cordotomies and midline postero-anterior myelotomies at various levels. They were able to demonstrate only a relative lateral to medial projection of lumbar and cervical components of the spinothalamic tract.

In man, Patrick (1893) was probably the first to follow the ascending fibres in the fasciculus of Gowers through the brain stem, but he traced them only as far as the region of the inferior quadrigeminal body. However, in cats he (1896) failed to find any degeneration above the pons after transection or hemisection of the spinal cord.

In 1896 Hoche described in the brain of a patient with an interruption of the spinal cord at C_9–C_{10}, fibres in the anterolateral fasciculus coursing cephalad as far as the inferior colliculus, where they turned back through the brachium conjunctivum to the cerebellum. Hoche apparently had no conception of a spinothalamic component in the ascending degeneration. Von Sölder (1897) also failed to follow the fibres in Gowers's fasciculus farther than the ventromedial border of the medial geniculate body. The credit for the first discovery of the termination of spinothalamic fibres in man should go to Quensel (1898). In a case with a spinal lesion at C_9–C_{10}, he not only traced the degeneration to the nucleus externus thalami, but also observed that fibres cross through the posterior commissure to the other side of the thalamus. Three years later Henneberg (1901) also described in man the termination of Gowers's fasciculus in the most posterior and ventral portions of the lateral nucleus of the thalamus. In 1910, Goldstein traced the degenerated fibres from the lower spinal cord of a man with a lumbar tumour to the ventral thalamus.

The difficulty in producing experimental degeneration of spinothalamic fibres above the pons in lower mammals and the uncertainty of staining technique, especially in human material, have delayed the understanding of this great afferent system for many years. Even with human material and modern techniques, demonstration of the termination of the spinothalamic fibres is not always successful. Gardner & Cuneo (1945), working on optimal material (from a fatality 21 days after anterolateral cordotomy), were puzzled by the scarcity of degenerating fibres near the thalamus. They suggested that spinothalamic fibres lose their myelin sheaths in the thalamus or that relatively few direct fibres reach the thalamus.

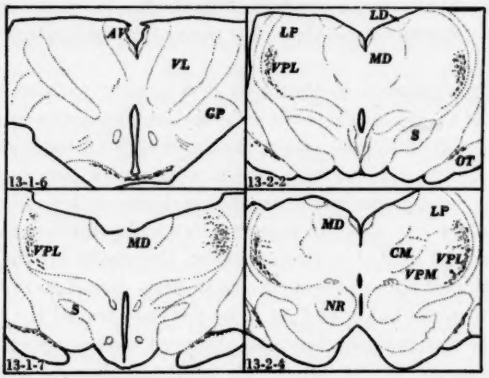
In an experiment on a cat, sacrificed 14 days after an anterolateral cordotomy in the second cervical segment, we have confirmed the observation of earlier workers that the degeneration cannot be traced above the pons.

However, in the experiments on spider monkeys we have demonstrated not only the existence of direct spinal fibres to the thalamus, but also a topographical arrangement of the fibres in the thalamic nucleus.

EXPERIMENTAL RESULTS

Experiment 1. *Spider monkey series no.* 13*; adult, male. Transection of the conus medullaris at* Ca_1 (1 *Sept.* 1944). *Sacrificed* 1 *Oct.* 1944. *Swank-Davenport preparations* (*Text-fig.* 1)

Lesion. The transection is histologically complete. Sections through the interrupted region of the cord show that the nervous tissue was completely destroyed and replaced by scar formation and disintegrated stroma-like materials including scattered black dust-like particles. There was no sign of infection or softening in any part of the cord.



Text-fig. 1. Sketches of serial sections showing the distribution of degenerated fibres in the thalamus after spinal transection at Ca_1 (SMS 13).

Histological examination of the thalamus. The degenerated fibres can be readily traced from the lesion through the spinal cord and into the brain stem. At the caudal border of the midbrain the degenerated fibres are grouped in an area just ventral to the round mass of the inferior colliculus where some fibres may be contributed to the parabigeminal body. Further cephalad they occupy the dorsolateral tegmental region. In the most cranial part of the midbrain a fraction of the degenerated fibres crosses in the dorsal division of the posterior commissure to the opposite side. Because of a gap in the serial sections, the exact course of these crossed fibres cannot be followed; but it is clear that none attains the pulvinar, though they approach that structure. The great majority of the degenerated fibres proceed in a loose cluster farther forward to the posterior end of the thalamus. Here they tend to be grouped along the lamina medullaris lateralis and then reach the dorsolateral part of the nucleus ventralis posterolateralis where they end. The terminations of the degenerated fibres, as manifested by fine, dark, dust-like particles, are limited to the dorsolateral

border of the nucleus. In the posterior thalamus some coarse fibres in the form of short irregular rods are seen coming from the medial aspect and usually making a sharp angle with fibres running along the lateral medullary lamina (Text-fig. 1).

Experiment 2. *Spider monkey series no.* 17*; one-year-old female. Spinal transection at* Ca_1 (28 *Nov.* 1944). *Sacrificed* 11 *Dec.* 1944. *Swank-Davenport preparations* (Pl. 1, fig. 1; Pl. 2).

Lesion. Sections at the level of the lesions show that no nervous tissue was left intact and the general outline of the cross-section of the spinal cord is unrecognizable. Microscopically the lesion appears as an irregular patch of black particles. However, the nerve roots surrounding the spinal cord which originate from higher segments are yellow in colour with occasional black granules and are little damaged.

Histological examination of the thalamus. The medulla and the posterior part of the midbrain of this animal were cut in longitudinal sections to gain a better orientation of the cerebellum.

Frontal sections of the thalamus were also made. This proved to be one of the best preparations so far as the degeneration in the thalamus is concerned. In the lowest sections from this block of tissue, spinothalamic fibres are seen grouped chiefly in an area between the corpus geniculatum mediale and the nucleus medialis mesencephali. They are dorsal to the medial lemniscus fibres and dorsomedial to the lateral lemniscus fibres. The majority of the degenerated fibres, after entering the posterior thalamus are crowded into the lamina medullaris lateralis which is entirely lateral to the field H_1 of Forel. The most conspicuous feature of the sections taken just above the superior colliculus is the crossing of a considerable number of degenerated fibres through the dorsal division of the posterior commissure (Pl. 1, fig. 1). These decussating fibres after separating from the spinothalamic tract, turn medially in a gentle curve to the opposite side. After crossing, they are directed towards the pulvinar, but do not enter that structure. By following the course of these crossing fibres section by section, it is clear that they are destined for the nucleus ventralis posterolateralis of the thalamus.

Anterior to the medial geniculate body, the degenerated fibres tend to be dispersed and to radiate laterally, but most of them are soon incorporated into the lateral medullary lamina along the ventrolateral border of the ventral nucleus. Some other fibres proceed farther forwards to the medial part of the ventral nucleus, then turn lateralwards and finally end in a very well-localized area in the dorsolateral border of the ventral nucleus. The latter fibres, which are partially intermingled with fibres crossing through the posterior commissure, are the cause of the scattered horizontally placed, short shreds of degeneration products in the posterior part of the thalamus. In some sections they appear to be in transit from the medial structures of the thalamus. Both the fibres taking this course and the fibres passing along the ventrolateral part of the

lateral medullary lamina terminate in a narrow strip at the dorsolateral border of the ventral nucleus (Pl. 2). The ventrolateral part of this nucleus seems to receive no terminating degenerated fibres, since decomposition products with the characteristic dust-like appearance of degenerated nerve endings are not present there in spite of numerous large degenerated fibres of passage.

In more anterior sections, the horizontally running fibres are no longer seen, but the small area of termination and the course of fibres from the lateral medullary lamina remains distinct.

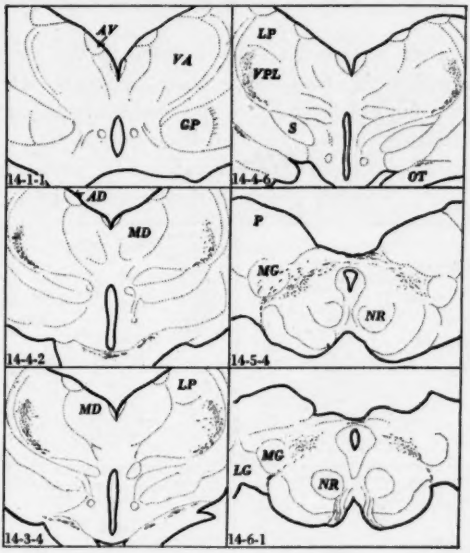
Experiment 8. *Spider monkey series no.* 14*; adult, female. Left hemisection at* Ca_1 (8 *Sept.* 1944). *Sacrificed* 26 *Sept.* 1944. *Swank-Davenport preparations* (Text-fig. 2; Pl. 1, fig. 2).

Lesion. The hemisection of the cord was complete except for a part of the sulcomarginal funiculus near the ventral median sulcus. On the right side the dorsomedial margin of the dorsal column was involved in the lesion.

Histological examination of the thalamus. The course of the degenerated fibres in the spinal cord and medulla have been described elsewhere (Chang & Ruch, 1947). At the level of the inferior colliculus the degenerating fibres stream dorsalwards along with the lateral lemniscus and a little medial to it. They then occupy a rather large area just ventrolateral to the oval nucleus of the inferior colliculus. In the anterior part of the midbrain they remain in the dorsal tegmental region. In the sections from the caudal end of the thalamus a considerable number of degenerated fibres pass through the dorsal half of the posterior commissure to the other side (Pl. 1, fig. 2). A large proportion of the degenerated fibres can be followed through the lateral medullary lamina to the nucleus ventralis posterolateralis of the thalamus. There are some short and horizontally directed fibres present in the posterior half of the ventral nucleus, which are apparently coming from the medial aspect of the thalamus. The terminal arborizations of the degenerated thalamopetal fibres, manifested by a characteristic ashy or dust-like appearance, are confined to a narrow and slightly curved strip bordering the dorsolateral sector of the nucleus ventralis posterolateralis. The boundaries of this area are rather sharp. In the ventrolateral border of this nucleus, especially at the level of posterior thalamus, a bunch of coarse degenerated fibres is present. Undoubtedly, these are fibres of passage since there is no sign of breaking up of the large osmium granules into fine splinters suggestive of degenerated nerve terminals.

On the other side of the thalamus degenerated fibres are distributed in the corresponding area of the nucleus ventralis posterolateralis of the thalamus, but two significant differences are worthy of mention. First, the number of the degenerated fibres is much greater in the right thalamus than might be expected in view of the great inequality in intensity of degeneration in the two sides of the spinal cord, medulla and midbrain. Secondly, the area of the right thalamus in which degenerated fibres terminate is not so restricted as on the

left (operated) side. The distribution of the degenerated fibres in the thalamus is shown in the sketches in Text-fig. 2.



Text-fig. 2. Sketches of serial sections showing the distribution of degenerated fibres in the thalamus after spinal transection at Ca_1 (SMS 14).

Experiment 4. *Spider monkey series no.* 18*; one-year-old male. Unilateral section of the ventrolateral funiculus at* C_3 *on the right side* (5 *Jan.* 1945). *Sacrificed* 26 *Jan.* 1945. *Swank-Davenport preparation* (Text-fig. 3).

Lesion. The operation was designed to produce a unilateral lesion restricted to the fasciculus of Gowers. Although the primary lesion was confined to the anterolateral funiculus, the fibres in the fasciculus of Flechsig also underwent Wallerian degeneration. Fortunately, the involvement of Flechsig's fasciculus does not affect the picture of degeneration in the thalamus.

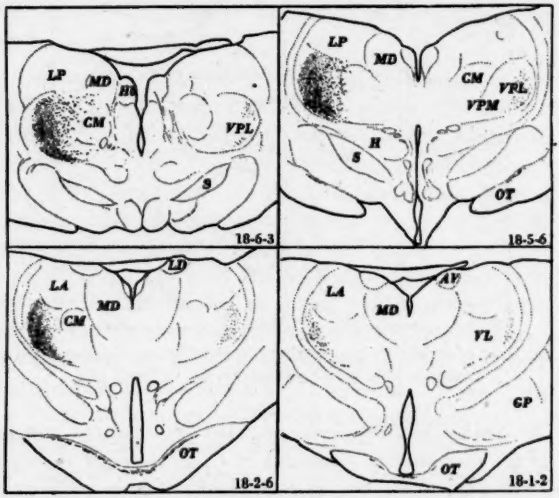
The area damaged by primary intention is shaped like a crescent with the concavity directed medially.

Histological examination of the thalamus. At the cephalic end of the midbrain the degenerated spinothalamic fibres are mingled with the undegenerated ones of the medial lemniscus system and occupy a large area between the medial geniculate body and the nucleus lateralis mesencephali. There is a gradual blending of the spinothalamic fibres with medial lemniscus fibres at this level and the topographical distinctness of the two systems becomes less and less clear, while the lateral lemniscus remains separated from them.

Degenerated fibres passing to the opposite side by way of the dorsal division of the posterior commissure are distinctly shown in many sections at this level;

the heavily myelinated ventral division of the commissure is completely free from degeneration. After crossing they continue laterally for a considerable distance toward the ventral border of the pulvinar, but none can be traced into that nucleus.

As the degeneration is traced farther forward to the posterior thalamus, a large number of widely dispersed degenerating fibres in the lateral region of the centrum medianum fan out laterally to the ventrolateral part of the ventral nucleus of the thalamus. Those elongated fragments of fibres are arranged in flat curves. The density of these fibres is greater in the posterior thalamus than in the anterior part. Another group of degenerated fibres is more densely



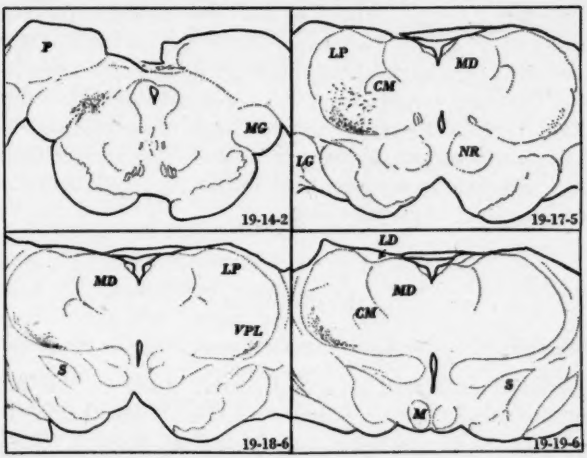
Text-fig. 3. Sketches of serial sections showing the distribution of degenerated fibres in the thalamus after a cordotomy on the right side at C_3 (SMS 18).

aggregated in the lateral medullary lamina and proceeds along the ventrolateral border of the ventral nucleus. The terminations of the degenerated fibres are distributed throughout the nucleus centralis posterolateralis of the thalamus. The most abundant disintegration products from the nerve terminals are found in the most lateral part of this nucleus. The apparent separateness of this lateral area of degeneration is enhanced by the presence of coarse fibres of passage. The size and arrangement of the elongated shreds of decomposition products in the centrum medianum and the nucleus arcuatus suggest that these nuclei may not constitute a terminus but are traversed by the spinothalamic fibres. The margin of the area with fine particles of degeneration at the dorsal aspect of the nucleus ventralis posterolateralis is well delimited from the nucleus lateralis (Text-fig. 3). Sections through the anterior part of the thalamus show that the degenerations do not extend beyond the oral end of the ventral nucleus.

The distribution of the degenerated fibres in the thalamus of the right side is similar in position, but much less dense than that in the left thalamus as described above.

Experiment 5. *Spider monkey series no.* 19*; adult, female. First operation: transection of the spinal cord at* Ca_1 (13 *July* 1945). *Second operation: left hemisection of the spinal cord between* L_1 *and* Th_{13} (10 *Jan.* 1946). *Sacrificed* 30 *Jan.* 1946. *Swank-Davenport preparation* (Text-fig. 4).

Lesion. The extent of the first lesion is not known since the caudal segments of the cord at Ca_1 was not sectioned for histological purposes. At autopsy that part of the cord was found shrunken and deformed. The permanent



Text-fig. 4. Sketches of serial sections showing the distribution of degenerated fibres after a left hemisection at I_1 6 months after total transection at Ca_1 (SMS 19).

paralysis of the tail guarantees the complete separation of the conus from the above structures. Sections taken just above the first lesion, i.e. at S_2, show that the ascending degenerations from the first operation have been largely cleared and the descending degenerations from the second lesion are limited to the left side.

The second lesion was confined to the left half of the spinal cord. The medial margin of the lesion is sharp and does not encroach on the other side. The hemisection is complete except for a very narrow strip along the median line which is a little distorted.

Histological examination of the thalamus. While ascending to the anterior region of the midbrain, the degenerating fibres on the left side are, as in all the other specimens, mainly grouped in the region between the dorsal tegmental region and the medial geniculate body, and are partly mingled with normal fibres of other origin. The location of the degenerated fibres is more dorsomedial

with reference to the medial lemniscus. Slightly higher up, the degenerated fibres are again present in the dorsal portion, and dorsal portion only, of the posterior commissure. After crossing, series of dark beads can be followed well laterally. In more anterior sections, the degenerated fibres enter the nucleus ventralis posterolateralis by two different routes, first, a medial-lateral approach from the region about nucleus arcuatus and the lateral half of the centrum medianum, and, second, an inferior approach by way of the ventrolateral border of the ventral nucleus. Although the coarse fibres are distributed to an extensive field, the area of termination seems to be limited to a small region in the ventrolateral sector of the nucleus ventralis posterolateralis. At the mid-thalamic level where the horizontally placed large fibres of passage are greatly reduced in number, the degenerations are clearly restricted to the lateral border of that nucleus (Text-fig. 4). The characteristic narrow band of degeneration with fine particles at the dorsolateral corner of the ventral nucleus, constantly present in all the other preparations, was apparently cleared of degeneration products. Thus Text-fig. 4 gives a negative picture of the thalamic terminus of fibres from the caudal region. In many sections the degenerated fibres are seen radiating successively in an arciform fashion from the lateral medullary lamina to the ventral nucleus, where they end.

DISCUSSION

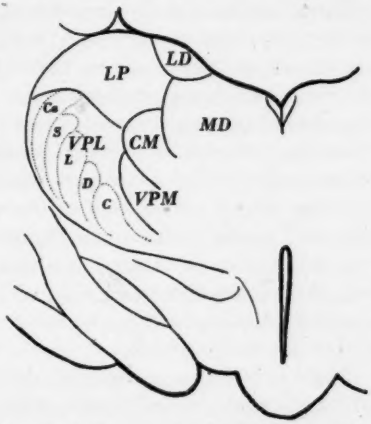
Topographical distribution of spinothalamic fibres in the thalamus

The topographical projection of spinothalamic fibres in the thalamus as revealed in the present investigation resembles closely that of the medial lemniscus system. As demonstrated by Le Gros Clark (1936) & Walker (1938*a*), the fibres arising from the nucleus gracilis, which receives impulses from the lower extremities, are projected rather to the nucleus ventralis posterolateralis and those from the nucleus cuneatus, relaying the nerve impulses from the arms, to the nucleus ventralis posteromedialis.

The degeneration in the thalamus of spider monkeys with a lesion at the caudal region of the cord is strictly confined to the most dorsolateral band of the nucleus ventralis posterolateralis and the medial portion of this nucleus was involved only when the spinothalamic tract was sectioned at a cervical level. The area of degeneration in the thalamus following a lumbar lesion does not extend medially as far as that produced by a cervical lesion. More crucial is the fact that the most dorsolateral region of the nucleus ventralis posterolateralis was free of osmium granules after a lumbar lesion performed six months after a total transection of the caudal segment of the cord. It is evident that the degeneration products of the spinothalamic fibres from the caudal cord had been cleared when the second operation was made, and no fresh degeneration in that area was produced. Therefore both positive and negative evidence demonstrates that the most dorsolateral corner of the nucleus ventralis

posterolateralis of the thalamus receives direct fibres from the caudal cord, and constitutes the tail area of that nucleus.

A comparison of the location and extent of the degeneration fields in the ventral nucleus of the thalamus following spinal lesions at three different levels suggests a precise segmental representation in which afferent fibres from the lowest part of the cord terminate in the most lateral zone of the nucleus ventralis of thalamus, and those from more cranial segments project successively to the more medial zones. These concentric zones of termination are so arranged that the lateral is more dorsally placed and the medial more ventrally. The sketch in Text-fig. 5 shows the plan of this topographical arrangement. In some normal myelin sheath preparations the loose bundles of very fine myelinated fibres from the ventral medullary lamina are seen arching dorsally in gentle curves into the ventral nucleus of the thalamus so that the nucleus is divided into several concentric laminae like those shown in Text-fig. 5. The outer two strands of arching fibres are usually more prominent than the inner, which are composed of fine myelinated fibres and are faintly stained in our specimens. This feature of the myeloarchitectonics of the thalamus has been noticed by Poliak (1932). He described concentrically arranged, semicircular laminae of thalamopetal fibres in the lateral (ventral) nucleus of the thalamus and pointed out the significance of this regular organization.



Text-fig. 5. Diagram showing the topographica arrangement in the thalamus of spinothalamic fibres from different levels of the spinal cord.

The plan of the topographical representation of various segments of the body in the thalamus as suggested by degeneration studies of spinothalamic fibres is consistent with that based on retrograde degeneration experiments and on physiological experiments.

Extensive studies of the thalamocortical projections of the thalamus have been made by Le Gros Clark (1936) and by Walker (1938*a*) who located the cells of the thalamus that undergo retrograde degeneration following ablation of different areas of the cerebral cortex. The superior segment of the postcentral gyrus receives projection fibres from the most lateral region of the nucleus ventralis posterolateralis, and the mid-postcentral area from the medial portion of that nucleus. From physiological and clinical evidence it is known that the superior portion of the postcentral gyrus is concerned with the somatic sensibility of the lower limb and the subjacent region of the postcentral gyrus is concerned with the arm. More medially still is the nucleus ventralis posteromedialis, which receives fibres from the trigeminal system and projects to the

inferior or face region of the sensory area; it is concerned with the sensibility of the face including taste (Blum, Walker & Ruch, 1943; Patton, Ruch & Walker, 1944).

The functional localization of the thalamus as investigated by the method of local strychninization by Dusser de Barenne & Sager (1937) is in agreement with the results of anatomical studies. Injection of a small amount of strychnine into the lateral region of the lateral and ventral nuclei of the thalamus produced sensory disturbances in the hind legs, whereas strychninization of the medial region produced disturbances in the arms.

The organization of the somatic afferent fibres at several levels of the nervous system seems to be determined by topographical principles. In 1900 Wallenberg first described an orderly termination of the secondary sensory neurons in the thalamus of rabbits. He proved that the so-called law of eccentric position of the long tracts postulated by Kohnstamm (1900) for Gowers' fasciculus is applicable to the thalamus as well as to the spinal cord and brain stem. The law states that the fibres originating most caudally occupy the most peripheral position, while the fibres arising from more cephalic segments of the spinal cord take an inner course in the ascending tract. The thalamus of the spider monkey, because of the extraordinary development of the tail region of the spinal cord, constitutes a special test of this theory. And the dorsolateral termination of fibres derived from the tail is exactly that predicted by the Kohnstamm-Wallenberg hypothesis. There seems no doubt that the primate thalamus, or at least the thalamus of the spider monkey, has been differentiated to such a degree that different segments of the body are represented in an orderly manner in rather restricted laminae of the ventral nucleus of the thalamus.

There is of course a considerable overlapping in the thalamus of the contributions from successive dermatomes. In view of the small cross-sectional area of the thalamus some overlapping of the dermatomic projection of the spinothalamic fibres in the thalamus is to be expected. The maximal projection area for each individual segment of the body may not necessarily be a thin sharply delimited strip. However, the thalamic fields for tail, legs, arms and face are definitely more sharply separated from one another in the sense that fibres from the same region of the spinal cord, i.e. sacro-caudal, lumbar or cervical, tend to be more closely grouped together and divided off by strands of fibres, whereas more overlapping exists within each sub-region. The thalamic overlapping of dermatomic contributions appears to be the anatomical counterpart of the overlapping of cortical fields for different segments of the cord as schematized by Woolsey, Marshall & Bard (1942). The trigeminal, cervical, thoraco-lumbar and sacro-caudal divisions are each represented in a specific region of the postcentral gyrus with the projection of each individual segment belonging to the same spinal division exhibiting a higher degree of overlapping.

Decussation of spinothalamic fibres in the posterior commissure

In all preparations from spider monkeys with spinal lesions degenerated fibres are constantly present in the dorsal part of the posterior commissure and pass laterally after decussation to the opposite thalamus. The crossing of the spinothalamic fibres through the posterior commissure has received little attention. In the literature only one mention of spinothalamic fibres crossing through the posterior commissure has been encountered (Quensel, 1898). Recently, Keene (1938) found in the monkey that degenerated fibres can be traced through the posterior commissure to the ipsilateral thalamus after the commissure was sectioned on one side near the midline. However, she was unaware of the spinal origin of these fibres.

The posterior commissure is actually a decussation rather than a true commissure. It is a collection of crossing fibres of various origin and termination. Besides the familiar components connecting the nucleus of Darkschewitch with the fasciculus longitudinalis medialis there are, according to Keene's analysis, at least five other components in the posterior commissure, namely fibres running to or in: (1) the tegmental region and the capsule of the red nucleus; (2) the habenular ganglia and the fasciculus retroflexus Meynerti; (3) the pineal body; (4) the corpus striatum or even the cortex; and (5) the thalamus.

Histologically, the posterior commissure is divided into two parts, a dorsal and a ventral. The ventral part is composed of coarse myelinated fibres. Fibres in the dorsal part are fine and run laterally for a considerable distance. The degenerations resulting from spinal lesions were always limited to this fine-fibred dorsal part of the commissure. According to Muskens (1914) the horizontal laterally coursing fibres may go to the globus pallidus, but in our sections degenerated fibres cannot be followed that far.

The problem of ipsilateral sensory representation

The discovery of crossed spinothalamic fibres in the posterior commissure serves to reconcile certain discordant functional and anatomical observations. It is commonly taught that each thalamus is linked only with the contralateral side of the body by the spinothalamic tract and the medial lemniscus system, and that the spinothalamic fibres cross immediately after their origin in the spinal cord. The bilateral connexion of the spinothalamic tract with the thalamus by an additional, supraspinal decussation has been overlooked since Quensel's brief mention of it (1898). For example, Walker (1938*a*) observed but was unable to account for the degeneration in both thalami following a hemisection of the cord and concluded with reservations that both thalami receive fibres from one spinothalamic tract.

A bilateral representation of cutaneous sensibility in the thalamus and cortex has been experimentally established by Dusser de Barenne & Sager

(1937) and confirmed for the face by Woolsey, Marshall & Bard (1942) with electrical techniques. Strychninization of one cortex or of one thalamus produces sensory disturbances in the skin of both sides of the body with the strongest symptoms manifested on the contralateral side. Dusser de Barenne pointed out that such bilaterality is not due to interhemispheric connexions between the sensory cortex of the two sides, since bilateral symptoms persisted after extirpation of one hemisphere. Moreover, anatomical search for a crossed connexion between thalamus and cortex has been unsuccessful, therefore the crossing of the sensory impulses must occur below the thalamic level.

Clinical investigation by Déjerine & Roussy (1906) also suggests a bilateral representation of cutaneous sensibility in the thalamus. Destructive lesions of the ventrolateral nucleus of the thalamus result in severe hypo-aesthesia and hypo-algesia and disturbances in deep sensibility. However, cutaneous sensibility to some degree recovers while deep sensibility is permanently lost. The recovery of cutaneous sensory function must depend upon the persistence of intact fibre connexions of the remaining thalamus with the two sides of the body. The present finding that the spinothalamic tract of one side reaches both thalami by way of fibres crossing in the posterior commissure provides an anatomical basis for bilateral cutaneous sensory representation of thalamus and cortex.* The permanent loss of deep sensibility on one side of the body after the unilateral destruction of the thalamus is accounted for by the single and complete decussation in the medulla of the fibres of the medial lemniscus system which is concerned with the conduction of proprioceptive sensibility.

SUMMARY

1. A topographical distribution in the thalamus of the spinothalamic fibres from three levels of the spinal cord has been demonstrated on the basis of Marchi preparations from five spider monkeys.

2. The spinothalamic fibres terminate exclusively in the nucleus ventralis posterolateralis in a laminated fashion so that fibres from the caudal segments of the cord are distributed to the extreme dorsolateral strip and those from the sacro-lumbar and the thoraco-cervical regions are distributed to successively more ventromedial laminae of this nucleus.

3. A part of the spinothalamic fibres cross through the dorsal portion of the posterior commissure and terminate in the nucleus ventralis posterolateralis of the opposite thalamus. These fibres are believed to form an anatomical basis for ipsilateral sensory representation in the thalamus and cortex.

* Foerster (1927) and others have suggested on the basis of cordotomy cases that some fibres of the spinothalamic system ascend the spinal cord on the side of entry, and such fibres could not be distinguished in our experiments from those of ventrolateral origin. However, with more radical cordotomies and more complete loss of pain sensibility, the hypothesis of an ipsilateral spinal pain pathway has lost favour. Thus it would seem that some fibres of the spinothalamic tract undergo double decussation.

ABBREVIATIONS

AD, nucleus anterodorsalis; *AV*, nucleus anteroventralis; *CM*, centrum medianum; *GP*, globus pallidus; *H*, H field of Forel; *Hb*, habenula; *LA*, nucleus lateralis anterior; *LD*, nucleus lateralis dorsalis; *LG*, corpus geniculatum laterale; *LP*, nucleus lateralis posterior; *M*, corpus mamillare; *MD*, nucleus medialis dorsalis; *MG*, corpus geniculatum mediale; *NR*, nucleus ruber; *OT*, tractus opticus; *P*, pulvinar; *S*, nucleus subthalamicus; *VA*, nucleus ventralis anterior; *VL*, nucleus ventralis lateralis; *VPL*, nucleus ventralis posterolateralis; *VPM*, nucleus ventralis posteromedialis.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Unretouched photomicrograph showing the decussation of spinothalamic fibres in the dorsal part of posterior commissure (SMS 17, spinal transection at Ca_1). 20×.

Fig. 2. Unretouched photomicrograph showing the degenerated fibres in the posterior commissure (SMS 14, spinal hemisection at Ca_1). 20×.

PLATE 2

Unretouched photomicrograph showing termination of degenerated fibres in the dorsolateral region of the nucleus ventralis posterolateralis of the thalamus (SMS 17, spinal transection at Ca_1). 70×.

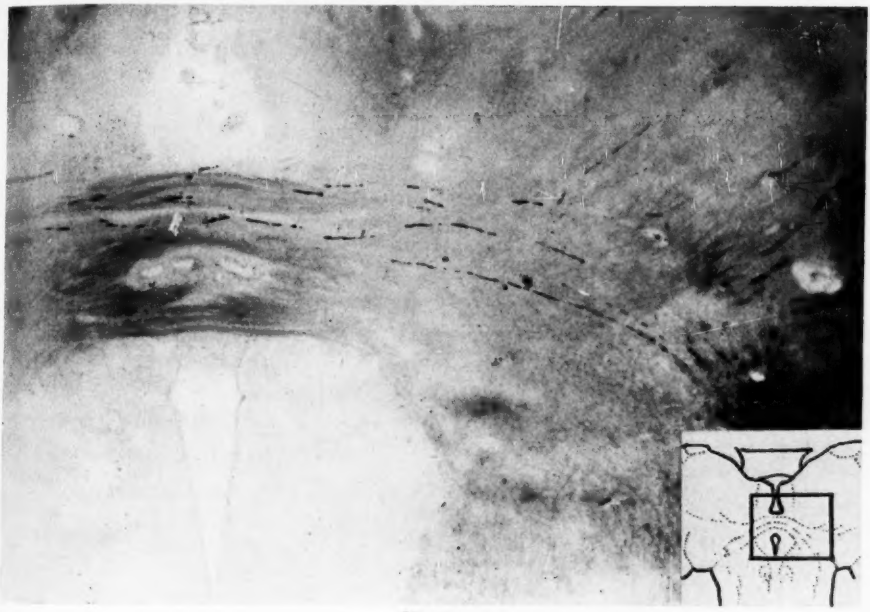


Fig. 1.

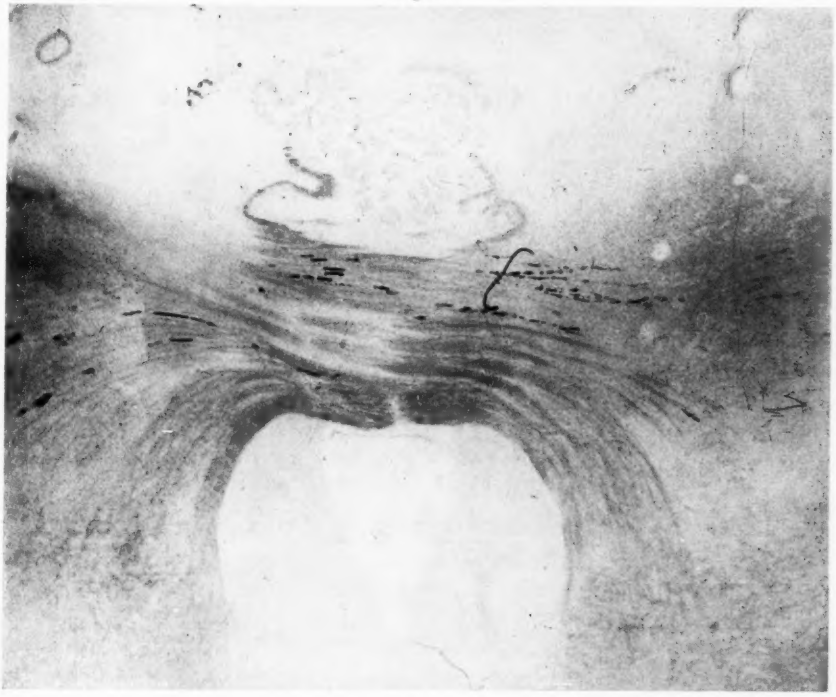
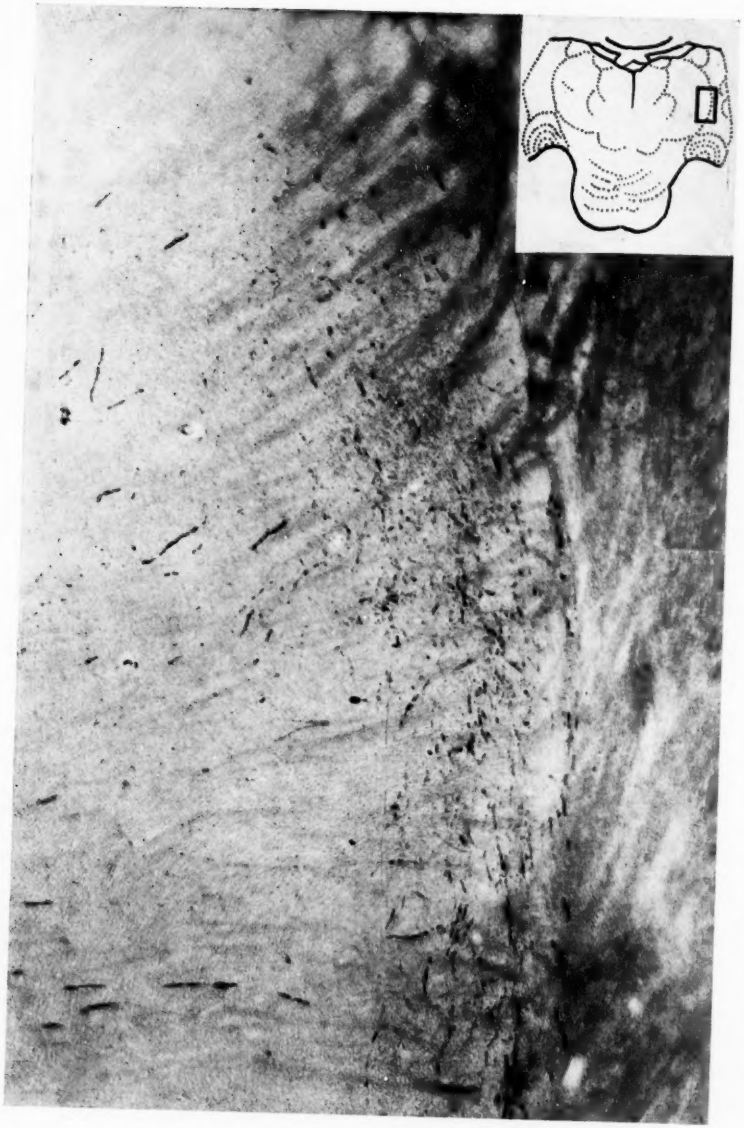


Fig. 2.

CHANG AND RUCH—SPINOTHALAMIC FIBRES IN THALAMUS OF SPIDER MONKEY



CHANG AND RUCH—SPINOTHALAMIC FIBRES IN THALAMUS OF SPIDER MONKEY

CRACKING IN THE METACARPO-PHALANGEAL JOINT

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INTRODUCTION

Joint cracks have always aroused the interest both of anatomists and of casual observers, yet no detailed investigation of their nature appears to have been made. Nordheim (1938) and Mennell (1939) have made important contributions on the radiological and physical phenomena associated with cracks; their conclusions are considered later, after a description of the various phases of a typical cracking joint. Detailed observations and measurements have here been restricted to the metacarpo-phalangeal joint of the medius, but other joints have been used for comparison.

THE PHASES OF A TYPICAL CRACK

A tension of about 5 kg., insufficient to produce a crack, applied to the metacarpo-phalangeal joint while the muscles crossing the joint are relaxed, leads to a separation of the bones by about 0·5 mm., sometimes by as much as 2·0 mm. A slight groove appears over the joint, and a gap can be felt between the bones. When, after exerting such a tension to separate the articular surfaces, the bones are suddenly forced together, a sharp knocking can be felt, and in favourable cases heard, by both subject and observer. The separation can be repeated indefinitely without altering the state of the joint, and will be spoken of as the phase of preliminary separation as opposed to the phase of rest.

Stronger tension results in the production of the crack. The bones are seen and felt springing suddenly apart, separating by a further 1–3 mm.; the crack is heard as a sharp report, and the skin groove becomes more conspicuous.

When, after cracking, tension is relaxed, the articular surfaces again make contact and the skin groove disappears; but with moderate tension, considerably less than that required to produce the crack, the bones become widely separated and the skin groove deep. Further direct tension on the joint will not produce a second crack; the joint is in a refractory phase. Very strong tension, up to the limit the subject can bear, separates the joint surfaces a little farther, to a maximum of about 4 mm.

The refractory phase can be prolonged indefinitely by continuous or intermittent tension on the joint. If, however, the joint is undisturbed it regains its ability to crack in about 20 min., and the whole cycle can be repeated. In our experience the minimum time recorded between two cracks produced by direct tension was 17 min., and never more than 22 min. rest was required before a second crack could be produced.

RADIOGRAPHIC OBSERVATION

A radiograph of a typical joint in the resting phase (Pl. 1, fig. 1) shows the bones separated by a gap of 1·8 mm., the combined thickness of the articular layers of cartilage covering the two bones. Under moderate tension (Pl. 1, fig. 2) the bones lie a little farther apart, in the phase of preliminary separation, but the tissues between them still appear homogeneous since synovial fluid cannot be distinguished radiographically from the solid tissues that surround it. The separation of the bones in other joints has been recorded radiographically by Mennell (1939), and its occurrence negatives the possibility that cracking could be due to the breaking of a cohesive film between the articular surfaces, since they are already separated by a macroscopic interval before the crack is heard.

A radiograph taken under moderate tension immediately after cracking (Pl. 1, fig. 3), confirms the wide separation of the bones in this phase, and shows a sharply outlined clear space in the interior of the joint cavity. This space is bounded proximally and distally by the articular cartilages, whose surfaces can now be distinguished quite clearly in the radiograph, while, laterally, it bulges evenly outwards between the bones. Further tension leads to some enlargement of the space (Pl. 1, fig. 4), and alteration in its shape as it spreads over the articular surfaces, so that it eventually becomes Ω-shaped in antero-posterior view, the slightly curved chord of the D corresponding to the surface of the head of the metacarpal and the convex limb to the concavity of the base of the phalanx. The superimposed flexor and extensor tendons now make a vague shadow across the space. Exsanguination of the limb by winding a tight rubber bandage round it from finger tips to shoulder does not affect the crack.

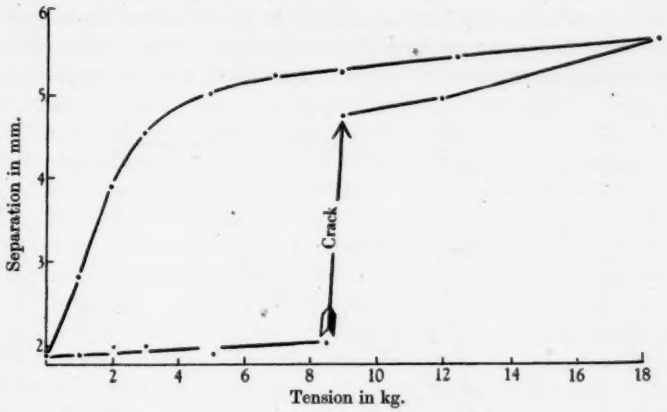
The appearance of spaces in joints under manipulation was noted by Fick (1911) in the metacarpo-phalangeal joint, but he did not discuss their nature, and their appearance in the normal living joint was immediately denied by Christen (1911). Dittmar (1932*a*) found that, in children, forces applied by pads and straps above and below the knee could lead to a separation of one or other of the femoral condyles from the surface of the tibia, and that the surfaces of the corresponding fibrocartilage were then outlined by a clear space. He and Felsenreich (1935), who repeated his work, were primarily interested in the possibility of photographing fibrocartilages for clinical purposes without the necessity of injecting the cavity with air, and neither author associated the spaces with cracking.

It was left to Nordheim (1938) to contribute an exhaustive and lavishly illustrated study on the spaces. He showed that they could be produced by suitable manipulations in most joints of normal individuals, whereas the earlier workers had supposed that the required separation of the bones could occur, in the adult, only in joints of which the ligaments had been weakened by disease. He was the first to associate the spaces in the metacarpo-phalangeal

joints with cracking, a point we can confirm, since we have always been able to demonstrate a space after a crack.

After the production of a crack, spaces can be seen when relatively low tensions are employed (Pl. 1, fig. 5), and the ease with which the bones can be separated in the refractory phase may be ascribed to the presence of these spaces. When no tension is applied in the refractory phase, and the articular cartilages are allowed to fall into contact, no spaces are visible (Pl. 1, fig. 6).

Occasionally multiple spaces are seen after manipulations involving repeated traction and relaxation (Pl. 1, fig. 7).



Text-fig. 1. Record of the separation of the bones in a typical cracking joint (J. B. R.).

MEASUREMENT OF TENSION

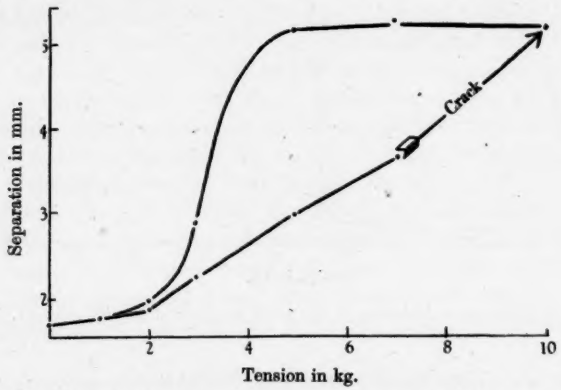
For quantitative investigation the subject was seated near a radiographic table; his middle digit was wrapped with adhesive plaster, and a stout string was tied tightly over this, round the proximal phalanx. The string was then attached through a spring balance to the upright member of the travelling carriage supporting the radiographic tube. The subject was instructed to draw his hand away from the upright till the balance, acting as a tensiometer, recorded the desired degree of traction. The tendons of the subject's finger were palpated to make certain that the muscles were relaxed, and an exposure was made. From measurements obtained from such a series of records taken at different tensions, before and after cracking, graphs were constructed. The number of exposures available from a single individual was limited by considerations of safety to about fifteen.

In a typical instance (J. B. R., Text-fig. 1) the bones were separated in the resting phase by 1·8 mm., and tensions up to 8·5 kg. led to a separation of about 2·0 mm. At this tension the joint cracked, and the next radiograph, taken at 9 kg., showed a separation of 4·7 mm. A large space was now present

and the thicknesses of the articular cartilages could be measured separately; each contributed 0·9 to the initial 1·8 mm. separation of the bones. Increase of tension to a maximum of 18·5 kg. led to a small increase in separation (5·6 mm. total).

The subsequent reduction of tension gave a graph of quite different shape, for the separation was still 5 mm. at 5 kg. tension whence it dropped smoothly to its starting point. The space was barely visible at 3·9 mm. separation and no longer detectable at 2·8 mm.

Other graphs of cracking joints showed variations in details, particularly in the degree of separation of the bones before cracking. In J. K., for instance, there was a separation of 3·7 mm. between the bones before cracking (Text-fig. 2). However, the curves have the same general form, a discontinuity always occurring at cracking and always associated with the appearance of a space.



Text-fig. 2. Record of the separation of the bones in a cracking joint showing wide preliminary separation (J. K.).

NATURE OF THE SPACES

Dittmar (1932*b*) suggested that, since he had introduced no air into the joints he had examined, the spaces observed could not be gaseous, but represented accumulations of synovial fluid which became visible in the radiographs, owing to the difference in specific gravity between the fluid and the neighbouring tissues. Felsenreich (1935) could not agree that this was possible. Nordheim (1938) investigated the point in the cadaver, and he found that a wrist joint subject to tension showed a space, but that the injection of water caused the space to disappear at once. He suggested accordingly that the space was a partial vacuum occupied by water vapour and gases under reduced pressure, and that the similarity between his preparations and those produced by the injection of air into joints was due to the similar radiographic properties of vacua and gas-filled cavities.

Nordheim compared the joint space to that which appeared in a hypodermic syringe half-filled with water when the entry was blocked and the plunger pulled back with sufficient force. Such partial vacua can be studied more conveniently in a van Slyke apparatus, and in either case the space appears when the pressure is reduced to the vapour pressure of water. In the joint, however, conditions are different, for the cross-sectional area of the fluid through which tensions are transmitted before cracking measures but 2 sq.cm. A tension of about 7 kg. is required to produce a crack, thus subjecting the fluid to a tension of $3\frac{1}{2}$ kg./sq.cm. Atmospheric pressure is about 1 kg./sq.cm., so that at the time the joint cracks the pressure in the cavity is minus $3\frac{1}{2}$ atm. on the relative scale ($-2\frac{1}{2}$ A. on the absolute scale).

The ability of columns of fluids such as water to withstand considerable longitudinal pulls without separation of their molecules, thus exhibiting an inherent tensile strength, is seldom seen in laboratory practice, for under ordinary conditions liquids boil as soon as the pressure within them falls to their vapour pressure. But when a fluid contains no unwetted dust particles, and is contained in a perfectly wetted vessel (conditions usually reached experimentally after temporary subjection to pressures of the order of 1000 atm., which drive all gas nuclei into solution), there are no foci left about which spaces can form. Water after such treatment can be heated to 200° C. without boiling and will support considerable tensions. Moreover, treated solutions of bicarbonates and acids can be mixed without frothing (Dean, 1944; Harvey, Whitely, McElroy, Pease & Barnes, 1944). Tall trees depend on this tensile strength of fluids, which is unaffected by dissolved gases, to draw up the sap, through the vessels, to the leaves (Dixon, 1938).

In animals the tensile strength of the body fluids has not been recognized as being of particular importance, but the presence of gas nuclei has been studied as they affect the appearance of bubbles after decompressions. They are absent from the interior of most cells, and unicellular organisms such as *Euglena, Paramoecium* and *Amoeba* do not develop bubbles in their protoplasm even after decompression from 100 atm. But bubbles form far more readily on the surfaces of the cells and are known to be responsible for the joint symptoms in caisson disease (Harvey, Barnes, McElroy, Whitely & Cooper, 1944). In man it is generally accepted that atmospheric pressure is of some importance in the prevention of sudden dislocations of joints, and now it may be added that the intrinsic tensile strength of the joint fluid, as it lies in the space whose walls it wets, may double or treble the passive cohesive strength resisting such dislocations.

Once a space has been formed in a liquid it can be expanded indefinitely by a traction sufficient to overcome atmospheric pressure, and Nordheim's (1938) comparison with conditions in a hypodermic syringe is now justified. Nordheim had no method of measuring the tensions he employed and did not realize the change of pressure in the interior of the joint as it cracked, from about

$-2\frac{1}{2}$ atm. to a little above the vapour pressure of water, a change which explains for the first time the sudden springing apart of the bones, and the setting up of vibrations in the tissues that are heard as the crack. Mennell (1939) ascribed the sound to the sudden tightening of the fibrous capsule about the joint and this may play a part; but he did not notice the spaces in his radiographs.

No explanation has been found in the literature of the inability to crack in the refractory phase. It may be suggested however that, when tension is relaxed after cracking, the large space contracts to a gas nucleus as does the space in the hypodermic syringe. Such a nucleus would be invisible in a radiograph, but on the renewal of tension would again expand, and would prevent the sudden decrease of pressure in a gas-free joint cavity, which is an essential preliminary to the production of the crack. The duration of the refractory phase after cracking would correspond to the time required for such a minute bubble to pass into solution. This suggestion conforms to the facts, though the difficulty of demonstrating the presence of this nucleus in the resting joint has not yet been overcome.

THE SYNOVIAL FLUID

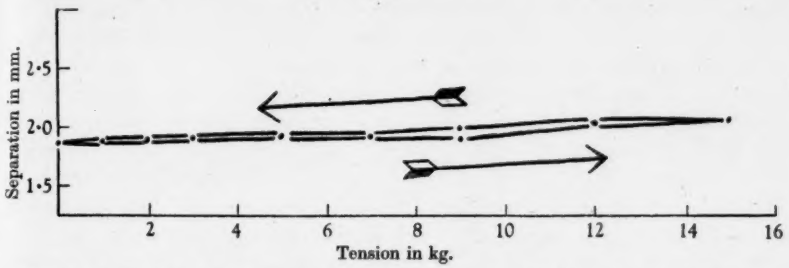
It is generally agreed that, apart from the mucin it contains, synovial fluid is a simple transudate, so that the pressure within the joint cavity should affect the rate of its formation. In the series of joints discussed in this paper, changes in the amount of fluid were kept at a minimum by maintaining tension across the joint for the shortest time compatible with achieving a steady tension and the making of the record.

Nordheim (1938), using continuous traction on the knee, found that after 2 min. the space was smaller and had broken up so as to appear like a string of pearls; after 10 min. no space remained, and the interior of the joint cavity appeared evenly dark in the radiograph, an effect he ascribed to a rapid inflow of synovial fluid obliterating the space. We have not been able to observe such massive inflows in the metacarpo-phalangeal joints, for after 10 min. continuous tension the space is still clear. Possibly the great extent of the synovial membrane of the knee, and its scantiness in the finger-joints, accounts for the differences observed.

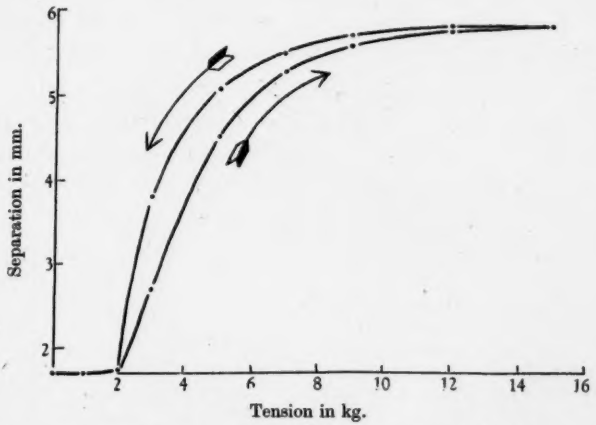
JOINTS WHICH DO NOT CRACK

The commonest cause of failure to produce a crack in the metacarpo-phalangeal joint is inability to relax the muscles whose tendons pass across the joint. But some individuals show a minimal separation of the bones at extreme tensions and inability to crack, though the tendons appear slack on palpation (Text-fig. 3). Possibly this is due to shortness of the collateral ligaments which would prevent a separation sufficient to produce a crack, for even prolonged pulls fail to separate the bones in these subjects to an extent comparable to that usually found.

In other subjects the bones separate easily under relatively light tension without cracking and without the appearance of a space. In such subjects remarkable degrees of hyperflexion and hyperextension are found, and the capsules and ligaments appear to be exceptionally lax, so that it is impossible to obtain the degree of tension in the synovial fluid required for cracking. In most subjects, in whom the metacarpo-phalangeal joint of the third digit cracks easily on direct tension, the corresponding joint of the fifth belongs to the lax class, and seldom cracks in response to direct traction.



Text-fig. 3. Record of the separation of the bones in a joint which did not crack (R. W. H.).



Text-fig. 4. Record of the separation of the bones in a joint which showed a space but did not crack (C. B. B. D.).

Another type shows wide separation and the presence of a space at relatively low tensions (4·5 mm. at 5 kg., Text-fig. 4 and Pl. 1, fig. 8). The course of the graph follows an even S-shaped curve from minimal to maximal tension, and the outward and returning tracks are close to each other. The behaviour of such joints is similar to that of other joints in their refractory phase and a space can always be demonstrated if the bones are sufficiently separated.

A joint, then, may fail to crack owing to either an inability to form a space at all or the too facile appearance of the space, so that the bones move apart smoothly instead of springing apart suddenly.

SUMMARY

1. In the resting phase the articular surfaces of the joint are in contact.

2. Light tension, up to about 6 kg., insufficient to produce cracking, leads to the phase of preliminary separation of the articular surfaces; recompression leads to reapposition of the surfaces with a sharp knocking sensation.

3. Stronger tensions, of about 7 kg. or more, lead to cracking. The noise is heard, the bones spring sharply apart, a groove appears in the skin over the joint, and a clear space appears in the radiograph within the synovial cavity.

4. The space is interpreted as a partial vacuum occupied by water vapour and blood gases under reduced pressure.

5. Measurement of tension indicates that the joint fluid sustains an absolute negative pressure of about $2\frac{1}{2}$ atm. before cracking occurs. As the space develops the pressure presumably rises to the vapour pressure of water.

6. After cracking, the joint is in a refractory phase and no further cracking can be elicited by direct tension for about 20 min. This phase is probably dependent upon the presence of a small gas nucleus in the fluid.

7. Inability to crack may be due to an inability to reduce pressure sufficiently in the joint cavity, or to the appearance of a space within the fluid at too low a tension.

Our thanks are due to Prof. A. B. Appleton, Drs J. F. Brailsford, J. Cyriax and J. W. McLaren for advice and assistance, and to our colleagues who have acted as subjects. Expenses were met by a grant from the Central Research Fund of the University of London.

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ROSTON AND WHEELER HAINES—CRACKING IN THE METACARPO-PHALANGEAL JOINT

EXPLANATION OF PLATE

Fig. 1. Resting metacarpo-phalangeal joint of medius (J. B. R.).
Fig. 2. At 7 kg. tension, before cracking, showing slight separation of the bones (J. B. R.).
Fig. 3. At 9 kg. tension, after cracking, showing the appearance of a space (J. B. R.).
Fig. 4. At 18¼ kg. tension, after cracking, showing the space enlarged (J. B. R.).
Fig. 5. At 5 kg. tension, after cracking, showing the space still visible at low tension (J. B. R.).
Fig. 6. Resting after cracking. No space visible in the resting joint (J. B. R.).
Fig. 7. At 9 kg. tension, after cracking, showing multiple bubbles (R. H. S. W.).
Fig. 8. At 3 kg. tension, showing space formed without cracking (C. B. B. D.).
Fig. 9. Effect of prolonged pull; separation without cracking or formation of a space (J. B. R.).

THE HISTOGENESIS OF THE EPIDERMIS IN THE RAT AND MOUSE

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INTRODUCTION

Although the epidermis of the mouse and rat are much used in cancer research their normal histogenesis has not been adequately described. Moreover, the lack of information about cellular differentiation in relation to cell division and stratification during the normal development of these tissues is particularly regrettable because the subject of cellular differentiation is of fundamental importance in cancer research. In this paper I have attempted to fill in these gaps in our knowledge. I have made a comparative study of the rat and mouse because, as is well known, the epidermis of the two species differs much in its sensitivity to certain carcinogenic agents (Glucksmann, 1945). In addition, I have studied epidermis from four different skin sites because Twort & Twort (1936) have shown that, in the mouse, various sites differ in their sensitivity to carcinogenic hydrocarbons. A quantitative method for following changes in the populations of differentiating, resting (undifferentiated), dividing and degenerating cells of human carcinomata during radiation treatment has been described by Glucksmann (1941). This method was developed after previous investigations at the Strangeways Research Laboratory on the developing eyes of tadpoles and rats both normal and irradiated (Tansley, Spear & Glucksmann, 1937; Spear & Glucksmann, 1938, 1941; Glucksmann & Spear, 1939; Glucksmann, 1940). The method has recently been applied (Glucksmann, 1945) to investigations of the normal post-natal development of mouse epidermis, of regenerative hyperplasia induced in mouse epidermis by chemical depilation and by turpentine painting, and of the changes following the single and repeated application of benzpyrene to mouse and rat skin. In the work reported in the present paper a modification of Glucksmann's method has been used to supplement the qualitative investigation of normal epidermal histogenesis of rats and mice in order to express more clearly the changes in the cell population during histogenesis and, secondly, to compare the results with those of Glucksmann (1941, 1945) already referred to.

Previous work. Previous investigations of epidermal development in rats and mice are summarized in Table 1 and will be discussed in relation to my own observations.

MATERIAL AND METHODS

Mus musculus L. var. *albinus* and *Rattus norvegicus* (Erxleben) were used. The specimens examined comprise: ten embryonic mice aged 10–18 days post-coitum; nine embryonic rats aged 11–22 days post-coitum; twelve mice and six rats aged 0–100 days post-partum. Animals of both sexes were used before puberty, but after puberty males only were selected, for Bullough (1943) has shown that, in the mouse, the thickness of the epidermis and the mitotic frequency of its cells undergo cyclic changes coincident with the phases of the oestrous cycle.

Table 1. *Summary of previous work on the histogenesis of the epidermis in mouse and rat*

References		Specimens	Sites
Steiner & Hitschmann (1927)	Rats	8-, 10-, 12-, 14-, 16-, and 18-day embryos	Various
Fraser (1928)	Rats	Series from 12th day post-coitum to 2nd day post-partum; also specimen 150 days old	Mid-dorsal
Erickson (1931)	Rats	Series from birth to maturity	Tail
Maurer (1892)	Mice	Embryo 18 mm. long	Not stated
Oyama (1904)	Mice	Embryo 21 mm. long	Not stated
Reed & Alley (1939)	Mice	Embryo 10 mm. long, about 15 days old	Not stated
Gibbs (1941)	Mice	Series from 0–15 days after birth	Anterior and posterior dorsal
Glucksmann (1945)	Mice	Series from 0–100 days after birth	Anterior dorsal

Epidermis from the following four regions was used (Text-figs. 1, 2).

(1) A rectangular area extending across the back from one forelimb to the other.

(2) A rectangular area extending across the back from one hindlimb to the other.

(3) A rectangular area extending across the ventral surface from one forelimb to the other.

(4) The under surface of the forefoot between the thenar and hypothenar pads.

The same four regions were used by Twort & Twort (1936). All tissues were fixed in Zenker's fluid (with 5% acetic acid). Sections were cut usually 8μ, occasionally 10μ, thick and were stained, in duplicate sets, some with Ehrlich's haematoxylin and others by a modification of Mallory's triple staining method in which carmalum replaces acid fuchsin.

PART I. QUALITATIVE OBSERVATIONS

The lack of a generally accepted terminology for epidermal strata makes it necessary to define the names used in this paper. The abbreviation 'S.' is used for 'stratum'.

S. germinativum: a basal layer of undifferentiated cells distinguishable from cells of the S. intermedium and S. spinosum by their smaller content of cytoplasm

and by having less distinct cell outlines and less conspicuous intracellular fibrils than the cells of the S. spinosum.

S. intermedium: a transitory embryonic stratum of undifferentiated cells which eventually become spinous cells with the development of intracellular fibrils.

S. spinosum: differentiating cells with relatively more cytoplasm, more conspicuous intracellular fibrils and more clearly defined cell outlines than the cells of the S. germinativum.

S. granulosum: cells containing keratohyalin granules.

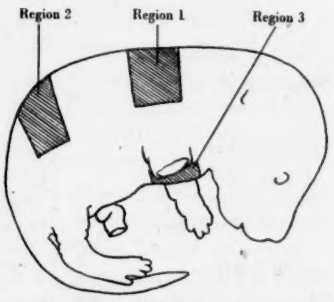
S. lucidum: cells containing eleidin.

S. corneum: fully differentiated (keratinized) cells.

S. disjunctivum: the disintegrating surface layers of the S. corneum.

Epitrichium: the keratinized layers which are shed when the first hair coat emerges.

Periderm: a transitory embryonic stratum, the most superficial in the epidermis, distinguishable from the S. germinativum and S. intermedium by its more flattened cells with denser cytoplasm.



Text-fig. 1. The position of regions 1, 2 and 3 on a 16-day mouse embryo.

It is necessary to point out that these various categories of cells, classified according to degree of cellular differentiation, are recognized by cytological criteria rather than by their location in the epidermis. In this paper the term 'differentiation' is used to refer only to the cytological changes leading to and involved in the process of keratinization.

(1) *The stages in histogenesis*

The developmental history of the epidermis can be arranged in stages according to the different strata present.

Stage A: S. germinativum only.

Stage B: S. germinativum and periderm.

Stage C: S. germinativum, S. intermedium and periderm.

Stage D: S. germinativum, S. spinosum, S. granulosum and periderm.

Stage E: S. germinativum, S. spinosum, S. granulosum, S. lucidum, S. corneum and periderm.

Stage F: S. germinativum, S. spinosum, S. granulosum, S. lucidum and S. corneum.

Stage G: S. germinativum, S. spinosum, S. granulosum, S. lucidum, S. corneum and, in region 4, S. disjunctivum.

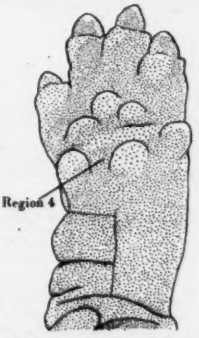
The various stages last for different periods, their duration differs also in the mouse and in the rat, and they persist for different times in different areas. For example, the duration of stages A, B and C is not equal over the whole

of regions 1–3. In the medial parts of regions 1–3 stages A and B end on the 13th and 14th days post-coitum in the mouse, and on about the 16th and 17th days in the rat. The particulars set forth in Table 2 deal only with the lateral parts of regions 1–3. The various stages are dealt with in more detail in association with the origin and development of each stratum.

No attempt has been made to give more than an approximate estimate of the duration of any of the embryonic stages of epidermal histogenesis: litters and even embryos within one litter vary in the rate of their development (Nicholas, 1932, for the rat; Grüneberg, 1943*a*, for the mouse).

(2) *The origin and development of each stratum*

The origin and development of each stratum will be described for the mouse, and the ways in which the rat differs from the mouse will be pointed out. Changes in the number of layers in each nucleated stratum (S. germinativum, S. intermedium, S. spinosum and S. granulosum) are shown in Table 2.



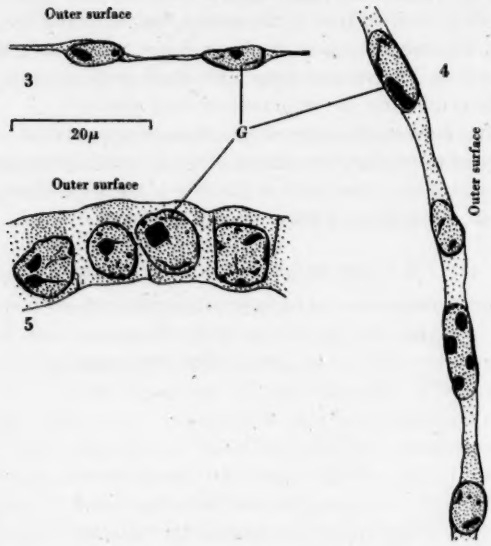
Text-fig. 2. The position of region 4 on the forefoot of a 16-day mouse embryo.

The *S. germinativum*, the deepest layer of the epidermis, is present in the youngest embryos (10-day mice and 11-day rats) and is responsible for the origin and maintenance of the other epidermal strata. Its cells are undifferentiated and in all specimens some are found dividing. In the mouse it consists of a single layer of cells which, in region 4, remains continuous at least up to 100 days after birth. In regions 1–3 it becomes incomplete by the 10th day post-partum: both spinous cells and the undifferentiated cells of the S. germinativum lie in the basal layer (Text-figs. 11, 12 and Pl. 2, figs. 10, 11), but small uninterrupted groups of undifferentiated cells remain in the immediate vicinity of the hair follicles. In the rat the S. germinativum is always a continuous layer.

In region 4 of all specimens the S. germinativum consists of columnar cells (Text-fig. 5) but in regions 1–3 the cells undergo several changes in shape during development. Until the 14th day post-coitum in the mouse and the 17th day in the rat they are flattened, in vertical sections appearing elongated parallel to the skin surface (Text-figs. 3, 4, and Pl. 1, fig. 1).

They are more flattened in region 3 than in regions 1 and 2 and in the medial parts of these three regions than in their more lateral parts. Similar regional differences in the shape of the cells of the S. germinativum have been described by Steiner & Hitschmann (1927) in 10- and 14-day rat embryos, by Fraser (1928) in 12-, 13-, 14- and 15-day rat embryos and by Steiner (1929, 1930) in early human embryos.

After the S. spinosum and S. granulosum have been formed the S. germinativum over the whole of regions 1–3 consists of columnar cells (Text-fig. 8 and Pl. 1, fig. 3). Slightly later, during the development of the hair follicles, these



Text-figs. 3–5. The epidermis of an 11-day rat embryo, stage A. 3. The medial part of region 1. 4. The lateral part of region 1. 5. Region 4. *G*=S. germinativum.

Table 2. *Stages (A–G) in the histogenesis of the layers of the epidermis (S. germinativum; S. intermedium; S. spinosum; S. granulosum; S. lucidum; S. corneum; periderm); the numbers of layers in each nucleated stratum; and the duration in days, post-coitum (–), post-partum (+), of each stage in mouse and rat (M and R). P=present.*

	Strata present and number of layers in each nucleated stratum								Time of ending of stages in days	
Stage	Germ.	Int.	Spin.	Gran.	Luc.	Corn.	Per.	Region	Mouse	Rat
A	1	—	—	—	—	—	—	1–4	10 –	12–15 –
B	1	—	—	—	—	—	1	1–4	13 –	16 –
C	1	1–3	—	—	—	—	1	1–4	14 –	18 –
D	1	—	3	1	—	—	1	1–4	16 –	19 –
E	1	—	2–1	4–3M 4R	P	P	1	1–3	0+	0+
	1	—	3	4	P	P	1	4		
F	1	—	1	3M 4R	P	P	—	1–3	Emergence of 1st pelage	
	1	—	3–2	4	P	P	—	4M		
G	1	—	2M 3–2R	3M 4–3R	P	P	—	4		
	1	—	1	3–2–3	P	P	—	1–3R		
	Incomplete	—	1– Incomplete	1 or 2– Incomplete	P	P	—	1–3M		

cells become cuboidal in the stretches of epidermis between the follicles but remain columnar in the vicinity of the follicles (Text-fig. 9); this has also been noticed by Fraser (1928). No subsequent changes in cellular shape have been observed. Intracellular fibrils appear in the cytoplasm on the 14th day post-coitum in the mouse and on the 18th day in the rat.

The *periderm*, the most superficial layer of the embryonic epidermis, is formed by the S. germinativum on the 12th day of embryonic life in the rat (Text-fig. 6 and Pl. 1, fig. 2): small numbers of periderm cells are already present in the 10-day mouse embryo, the youngest that has been examined. The periderm is formed first in region 4 and in the more lateral parts of regions 1 and 2; later it appears in region 3 and in the more medial parts of regions 1 and 2. Its absence over the neural tube in young rat embryos was noticed by Fraser (1928). The periderm persists almost till birth: it has been found in the oldest rat embryo, aged 22 days, but has not been identified in the mouse after the 17th day.

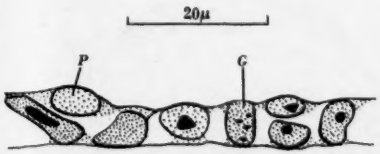


Fig. 6.

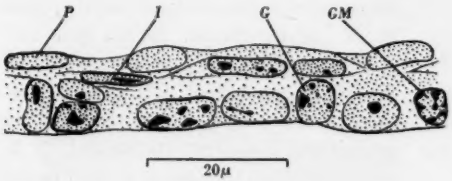


Fig. 7.

Text-fig. 6. The epidermis on the lateral part of region 1 of a 12-day rat embryo. Stage B. *P* = periderm.

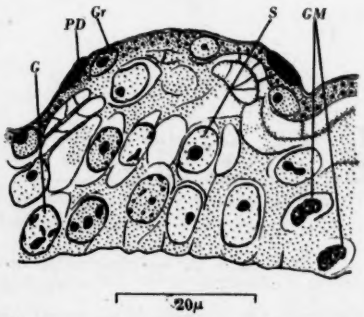
Text-fig. 7. The epidermis on the lateral part of region 1 of a 13-day mouse embryo. Stage C. *I* = S. intermedium. *GM* = dividing cell of S. germinativum.

For a short time after the periderm has been formed its cells are undifferentiated and able to divide (Pl. 1, fig. 2); cell division has ceased by the 14th day in the mouse and by the 17th day in the rat. Degenerate periderm nuclei (Text-fig. 8) are first noticed at these times and nuclei of normal appearance are no longer found after the 16th and 19th days in the mouse and rat respectively. Nuclear degeneration is accompanied by cytoplasmic changes, and the periderm soon has the appearance of a thin sheet of keratin with persistent nuclear remains (Text-fig. 9); this indicates that differentiation of the periderm probably takes place by parakeratosis as in human embryos (Pinkus, 1910). I have been unable to find, even in haematoxylin-stained sections, the basic-staining granules recorded by Fraser (1928) in the periderm of 18- and 19-day albino rat embryos.

The *S. intermedium*, situated between the S. germinativum and the periderm, is present by the 13th day of embryonic life in the mouse (Text-fig. 7) and by the 16th day in the rat. Like the periderm it is formed later in the medial than in the more lateral parts of regions 1–3; in one 13-day mouse embryo it is found in region 4 but is still absent on the trunk. Two observations indicate that the

S. intermedium is produced from the S. germinativum, viz. the cytological similarity of the cells in the two strata and the relatively small number of dividing periderm cells, probably sufficient only for increasing the surface area of the periderm and insufficient for contributing to the S. intermedium. The cells of the S. intermedium are undifferentiated and able to divide. In a short time it becomes about three cell layers thick and by the 14th day post-coitum in the mouse and the 18th day in the rat intracellular fibrils have been formed; the cells have begun to differentiate and the S. intermedium has become the S. spinosum.

The *S. spinosum*, when it is formed on the 14th day post-coitum in the mouse and on the 18th day in the rat, consists of three layers of cells. The number of layers decreases during development as shown in Table 2. On the trunk of the mouse the stratum becomes discontinuous after the 15th day post-partum, though small groups of spinous cells remain in the vicinity of the hair follicles above groups of undifferentiated cells of the S. germinativum. On the 10th day and afterwards some spinous cells are found intermingled with undifferentiated cells in the basal layer of the epidermis (Text-figs. 11, 12 and Pl. 2, figs. 10, 11). In region 4 of the mouse and in all four regions of the rat the S. spinosum and S. germinativum are always continuous strata.



Text-fig. 8. The epidermis on region 2 of an 18-day rat embryo. Stage D. *S* = S. spinosum. *Gr* = S. granulosum. *PD* = degenerating nucleus of periderm.

The S. spinosum is originally formed by the differentiation of the cells of the S. intermedium. A few dividing cells are found in the S. spinosum of region 4 until the time of birth (Pl. 1, fig. 6) but on the trunk cell division ceases earlier, by the 16th day in the mouse and by the 19th day in the rat. The cessation of cell division coincides with the time when the amount of cytoplasm is increasing and the intracellular fibrils are becoming more conspicuous. The division of spinous cells is insufficient for the maintenance of the S. spinosum, for which the S. germinativum is responsible. For a short time after the S. spinosum has been formed it is possible to distinguish between those spinous cells which have been produced by the differentiation of cells in the S. intermedium and those which have been produced by the S. germinativum; the latter, which lie deeper in the epidermis, are more polygonal in shape (Text-fig. 8).

Similarly in the early *S. granulosum* one can distinguish: (*a*) cells which are very flattened in shape, have small keratohyalin granules and have been formed by the differentiation of spinous cells of S. intermedium origin, and (*b*) cells which are less flattened, have larger granules and have never passed through a stage when they were constituents of the S. intermedium. The S. granulosum

is formed on the 14th day of embryonic life in the mouse and on the 18th day in the rat. Fraser (1928) first identified the stratum in a 20-day albino rat embryo, but it is possible that the basic-staining periderm granules which she described in younger embryos really belonged to the S. granulosum. As mentioned above, I have been unable to find such granules in the periderm; but keratohyalin granules are basic-staining and the very thin cytoplasm of the periderm is in many places difficult to distinguish in 18- and 19-day Norway rat embryos. Moreover Fraser enumerated 'six or seven' cell layers in the epidermis of an 18-day albino rat embryo and attributed only three or four of them to the 'S. intermedium' (corresponding to the S. spinosum of this paper) and S. germinativum; of the remaining three layers probably two belonged to the S. granulosum and one to the periderm.

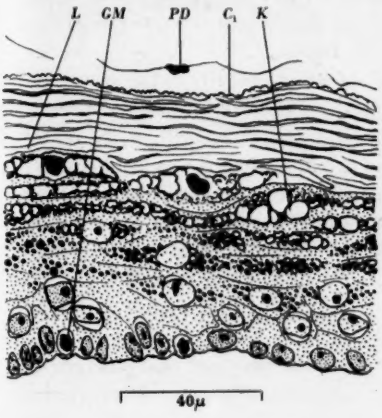


Fig. 9.

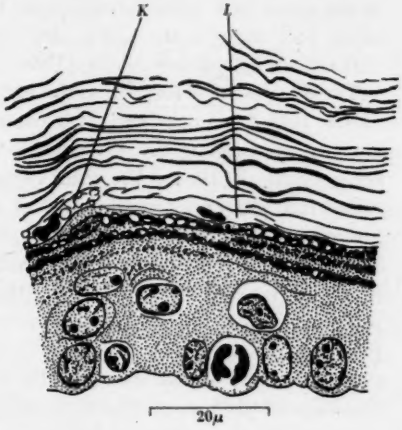


Fig. 10.

Text-fig. 9. The epidermis on region 1 of a 21-day rat embryo. Stage E. C_1 = S. corneum; K = large keratohyalin granule partly converted into eleidin; L = S. lucidum.

Text-fig. 10. The epidermis on region 1 of a mouse 3 days after birth. Stage F. Lettering as before.

Changes in the number of cell layers in the S. granulosum are shown in Table 2. On the trunk of the mouse the stratum has become discontinuous by the 20th day post-partum and is represented only by scattered cells containing small keratohyalin granules; they are found mainly in the vicinity of the hair follicles where there are also groups of undifferentiated cells and spinous cells. The stratum is never discontinuous on region 4 of the mouse or on any region of the rat. Dividing cells are never found in the S. granulosum; the nuclei in the oldest (most superficial) cells are usually degenerate.

The mode of formation of the *S. lucidum* by the differentiation of cells of the S. granulosum is indicated by the appearance presented by the most superficial cells (Text-figs. 9, 10 and Pl. 1, fig. 4) in the latter stratum. They contain a small number of very large granules probably formed by coalescence of the

smaller and more numerous keratohyalin granules of the deeper cells in the stratum. Each large granule has an outer wall of a substance giving the same staining reactions as the small keratohyalin granules; within the wall is a substance giving the same staining reactions as the cell contents of the S. lucidum, indicating that the conversion of keratohyalin to the eleidin of the S. lucidum begins in the centre of the granule. In some places in the S. lucidum it is possible to distinguish the outlines of granules which have been completely converted into eleidin and which have not yet become confluent with each other; in these places the S. lucidum cells are sometimes contiguous laterally with cells of the S. granulosum in which the granules still retain an outer covering of keratohyalin. The S. lucidum is first distinguishable on the 16th day post-coitum in the mouse and on the 19th day in the rat. It is thicker in the rat than in the mouse and, after the emergence of the first hair coat, on the lower surface of the foot than on the trunk. Its cells never divide and the few nuclei that still remain are all degenerate (Text-figs. 10, 13).

The *S. corneum* is formed on the 16th day of embryonic life in the mouse and on the 19th day in the rat. It is composed of horizontally arranged sheets of keratin representing the converted peripheral parts of the cells of the underlying strata. In the rat the outer surface of the first-formed sheet of keratin is raised into numerous small domes of about the same size as the keratohyalin granules in the outer cells of the S. granulosum (Text-fig. 9 and Pl. 1, fig. 4). This sheet of keratin can still be detected on the outer surface of the S. corneum up to the time of birth, indicating that no layers are shed from this stratum until after birth. The superficial sheet of keratin in the mouse lacks this peculiarity.

From its first appearance until the time when the first hair coat emerges the thickness of the S. corneum increases and it consists of relatively closely packed sheets of keratin in which it is difficult to distinguish the lateral cell boundaries (Pl. 1, fig. 5). On the lower surface of the foot it retains this structure for so long as its development has been studied (Text-fig. 13). On the trunk, however, the early S. corneum, or epitrichium, is shed when the first hair coat emerges.

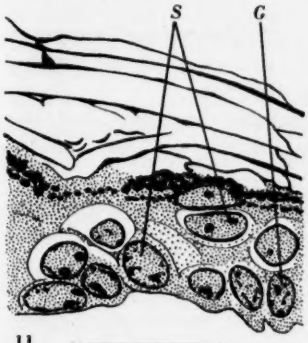
The sheets of keratin in the new S. corneum which replaces the epitrichium lie less close together and form a reticular pattern (in vertical sections) because the lateral boundaries of the cornified cells are visible (Text-fig. 11 and Pl. 2, fig. 9). These lateral boundaries usually lie vertically above each other and often above the junctions between adjacent cells in the outer layers of the S. granulosum. Fragments of the epitrichium are found outside the new S. corneum for some time after the hairs have emerged (Pl. 2, fig. 9). The new S. corneum of the mouse is considerably thinner than that of the rat.

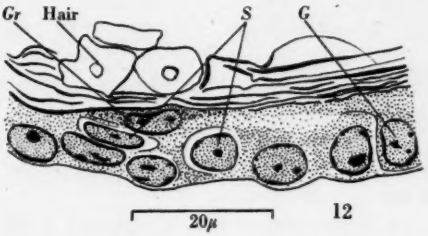
A *S. disjunctivum* has been identified only after birth and on the lower surface of the foot; it is distinguished from the rest of the S. corneum by small differences in staining reaction and by the fact that the sheets of keratin are broken and are more widely separated from each other.

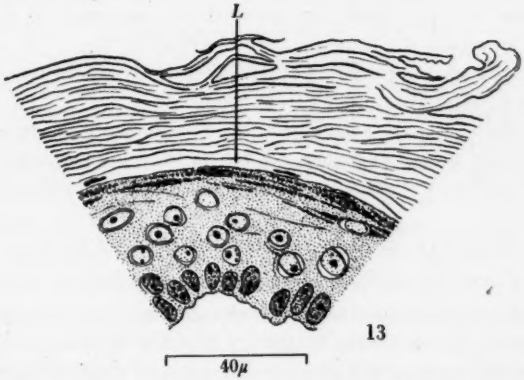
(3) *Summary of qualitative specific and regional differences*

Specific differences

(1) During embryonic life the rat enters each stage of epidermal histogenesis two to four days later than the mouse; this difference is correlated with the fact that the gestation period is about four days longer in the rat than in the mouse (Donaldson, 1924, p. 20; Grüneberg, 1943*b*, p. 6).







Text-figs. 11–13. Stage G. 11. The epidermis on region 3 of a mouse 10 days after birth. 12. The epidermis on region 3 of a mouse 100 days after birth. 13. The epidermis on region 4 of a mouse 29 days after birth. Lettering as before.

(2) At equivalent stages of development the reductions which take place in the number of cell layers in the S. spinosum and S. granulosum of region 4 and in the S. granulosum of regions 1–3 begin later in the rat than in the mouse (Table 2).

(3) The decrease in the thickness of the epidermis on the trunk after the emergence of the first hair coat proceeds further in the mouse than in the rat; and in the mouse, but not in the rat, the S. germinativum, S. spinosum,

S. granulosum and S. lucidum become disorganized and incomplete. Henceforward the S. corneum is thinner in the mouse than in the rat.

Regional differences

(1) During the early stages of epidermal histogenesis, on the medial as compared with the more lateral parts of regions 1–3 the cells of the S. germinativum are more flattened, and the periderm and the S. intermedium are formed later.

(2) The cells of the S. germinativum in region 4 are columnar in all specimens, even in the youngest embryos, whereas in regions 1–3 they are at first flattened and then cuboidal and do not become columnar until later.

(3) During the development of the first hair coat there are established other differences between the epidermis on region 4, where hairs are absent, and on the epidermis of the trunk (regions 1–3).

(*a*) The cells of the S. germinativum in region 4 remain columnar whereas in regions 1–3 they become cuboidal in the epidermis away from the vicinity of the hair follicles.

(*b*) In the spinous cells of regions 1–3 cell division ceases very soon after the formation of the S. spinosum and at the same time the amount of cytoplasm increases and the intracellular fibrils become more conspicuous. In the spinous cells of region 4 these changes do not take place until about the time of birth.

(*c*) When the first hair coat has emerged the epidermis in regions 1–3 is thinner than in region 4 (Table 2) and a different type of S. corneum has replaced the epitrichium. In region 4, however, the original type of S. corneum persists and a S. disjunctivum can be recognized.

(*d*) In the mouse the S. germinativum, S. spinosum, S. granulosum and S. lucidum remain intact in region 4 whereas they become disorganized and incomplete in regions 1–3 after the emergence of the first hair coat.

PART II. QUANTITATIVE OBSERVATIONS

(1) *An analysis of the cell population*

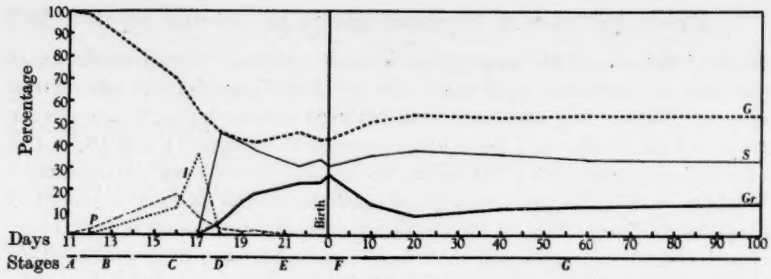
An analysis of the cell population of the developing epidermis has been made by a modification of the method used for biopsies of irradiated human carcinomata (Glucksmann, 1941) and for normal and treated mouse epidermis (Glucksmann, 1945) in which four categories of cells are counted: 'resting' (undifferentiated), degenerating, dividing, and differentiating. In the developing epidermis of the rat and mouse five categories of cells have been distinguished by the cytological criteria listed above: cells of the S. germinativum, S. intermedium, S. spinosum and S. granulosum, and cells of the periderm. In each specimen the numbers of nuclei belonging to cells in each of these five categories have been counted at all focal levels on several carefully selected rectangular areas of vertically sectioned epidermis away from the vicinity of hair follicles. The results have been expressed as percentages of the total number (usually about 500) of nuclei counted.

In Text-figs. 14–17 the percentage of each category of cells in the total population of nucleated epidermal cells is plotted against the age of the specimen. All four skin sites have been analysed separately but the results for regions 1–3 are similar and have been averaged (Text-figs. 14 and 16 for the rat and mouse respectively). The results for region 4 have been given separately (Text-figs. 15, 17). As previously mentioned the medial parts of regions 1–3 differ from the lateral parts in the youngest embryos, and in each of these cases the nuclei of all epidermal cells have been counted in each of several sections taken across the whole width of the region. The age of each embryo is usually known only to within ± 12 hr. of the approximate age, expressed in days, which has been assigned to it; moreover, as already mentioned, litters, and even embryos within one litter, vary in the rate of their development so that the embryos of the same approximate age may have reached different stages of epidermal histogenesis. For the purpose of spacing the embryos in drawing the graphs each has been assigned an age, expressed in days and fractions of days (e.g. 13·6 days), after consideration both of its approximate age and of the stage reached in epidermal histogenesis.

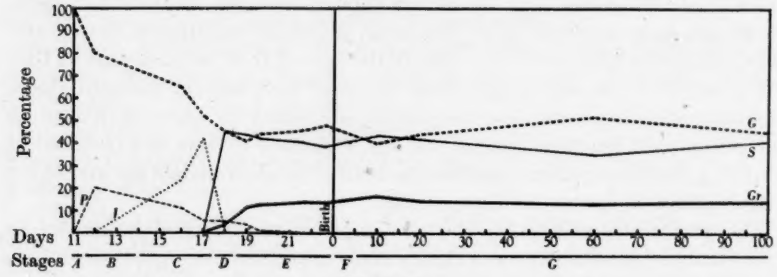
The graphical presentation of the changes in the cell population during epidermal histogenesis expresses clearly many of the features already described, such as: the time of formation of each of the nucleated strata and, in the case of the periderm and the S. intermedium, the time of its disappearance, and the combination of strata which helps to characterize the stages (A–G) of epidermal histogenesis. Moreover, the graphs demonstrate the changes in the numerical proportions of the different kinds of *cells* in the developing epidermis, whereas the qualitative observations could reveal only the developmental history of the *strata*. They show that for specimens at the same stage of epidermal development the proportion of undifferentiated cells (cells of the S. germinativum) to differentiating cells (cells of the S. spinosum and S. granulosum) is approximately the same in both the rat and the mouse. This indicates that cellular differentiation proceeds in the same manner in both species even though on the trunk of the mouse stratification of the epidermis disappears soon after birth.

During early embryonic development there is a conspicuous decrease in the percentage of undifferentiated cells and a proportionate increase in the percentage of differentiating cells. Using a similar quantitative method for analysing the cell population, Glucksmann (1945) has shown that during experimental carcinogenesis of the interscapular epidermis of the adult mouse the percentage of undifferentiated cells increases and the percentage of differentiating cells decreases, i.e. the reverse of the changes which take place during the development of the epidermis. He has also shown that when human carcinomata are successfully irradiated the percentage of undifferentiated cells decreases and the percentage of differentiating cells increases (Glucksmann, 1941).

The analyses of the cell population of the developing epidermis of rats and mice lead to conclusions similar to those reached at the end of the report on



Text-fig. 14.



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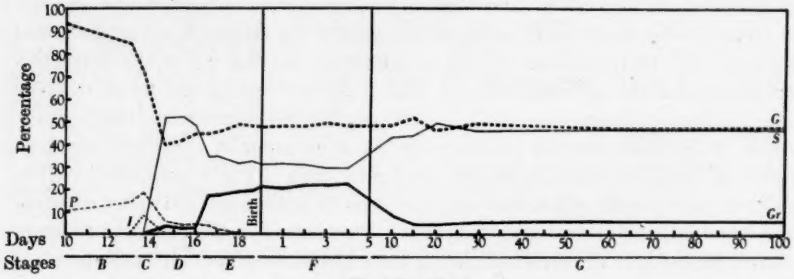
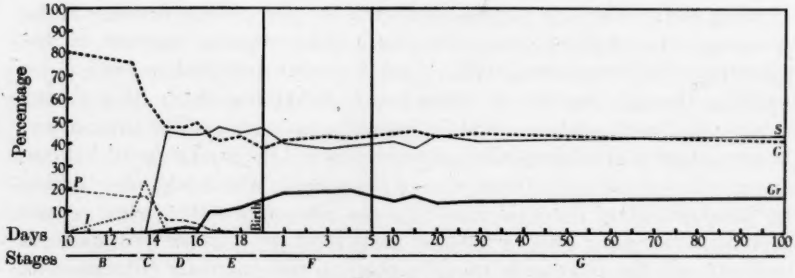


Text-fig. 16.



Text-fig. 17.

Text-figs. 14–17. The percentage numbers of five categories of cells in the total population of nucleated epidermal cells. Thick broken lines: S. germinativum; thin broken lines: periderm; dotted lines: S. intermedium; thin continuous lines: S. spinosum; thick continuous lines: S. granulosum. 14. Rat, average of regions 1–3. 15. Rat, region 4. 16. Mouse, average of regions 1–3. 17. Mouse, region 4.

qualitative observations. The most striking differences between the rat and the mouse are found on the trunk after the emergence of the first hair coat; the three trunk regions within each species closely resemble one another and differ from region 4, the differences becoming pronounced during the development of the first hair coat.

(2) *Dividing cells*

During the analysis of the cell population of the developing epidermis dividing and non-dividing cells in the S. germinativum, S. intermedium, S. spinosum and

Table 3. *The percentage numbers of four categories of dividing cells in the total population of nucleated epidermal cells. M, mouse; R, rat. The ages are given in days after copulation* (–) *or after birth* (+).

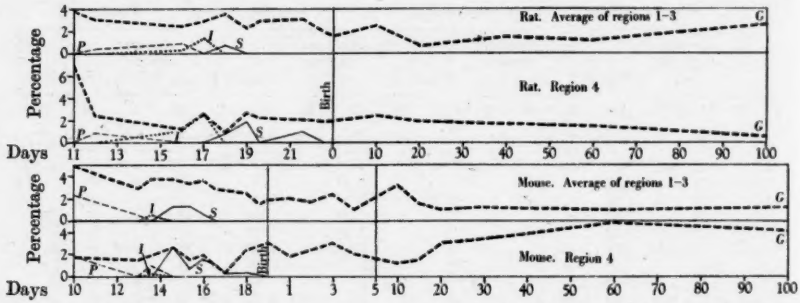
	S. germinativum				S. intermedium				S. spinosum				Periderm			
	Average regions 1–3		Region 4		Average regions 1–3		Region 4		Average regions 1–3		Region 4		Average regions 1–3		Region 4	
Age (days)	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R
10–	4·96	—	1·74	—	0	—	0	—	0	—	0	—	2·27	—	1·74	—
11–	—	3·81	—	7·05	—	0	—	0	—	0	—	0	—	0	—	0
12–	—	3·12	—	2·50	—	0	—	0	—	0	—	0	—	0·18	—	0·83
13–	2·99	—	1·53	—	0	—	0	—	0	—	0	—	0·46	—	0	—
13–	3·79	—	1·95	—	0·53	—	0·19	—	0	—	0	—	0	—	0·97	—
14–	3·82	—	2·64	—	0	—	0	—	1·29	—	2·64	—	0	—	0	—
15–	3·44	—	1·53	—	0	—	0	—	1·31	—	0·77	—	0	—	0	—
16–	3·71	2·53	1·96	1·30	0	0·32	0	1·11	0·56	0	1·57	0	0	0·84	0	0
16–	2·87	—	1·06	—	0	—	0	—	0	—	0·89	—	0	—	0	—
17–	2·81	2·95	0·38	2·61	0	1·38	0	2·61	0	0	0·38	0	0	0	0	0
18–	2·57	3·61	2·41	0·99	0	0	0	0	0	0·70	0·37	0·79	0	0	0	0
18–	1·58	—	—	—	0	—	—	—	0	—	—	—	0	—	—	—
19–	—	2·35	—	2·72	—	0	—	0	—	0	—	1·88	—	0	—	0
19–	—	2·97	—	2·33	—	0	—	0	—	0	—	0	—	0	—	0
21–	—	3·18	—	2·12	—	0	—	0	—	0	—	0·96	—	0	—	0
22–	—	1·96	—	1·98	—	0	—	0	—	0	—	0	—	0	—	0
0+	1·91	1·61	3·09	—												
1+	2·12	—	1·86	—												
2+	1·75	—	—	—												
3+	2·45	—	3·08	—												
4+	1·16	—	2·10	—												
10+	3·26	2·58	1·23	2·53												
15+	1·62	—	1·57	—												
20+	1·10	0·76	3·13	1·97												
29+	1·29	—	3·42	—												
40+	—	1·52	—	—												
60+	1·01	1·19	4·86	1·55												
100+	1·32	2·60	4·15	0·56												

periderm were recorded separately. The results of the observations on dividing cells are given in Table 3 and Text-fig. 18. The mitotic period is defined as extending from the earliest recognizable prophase to the latest recognizable telophase.

As might be expected the aggregate percentages of the various categories of dividing cells in the total population of nucleated epidermal cells is, on the whole, higher during embryonic life than after birth except in region 4 of the mouse. After the 15th day post-partum the percentage of dividing cells in the mouse is considerably higher in region 4 than on the trunk and also exceeds the percentage of dividing cells in any of the four skin regions of the rat. The

difference between the average mitotic indices of region 4 and region 1–3 of the mouse after the 15th day post-partum is statistically significant. The difference between the average mitotic indices of region 4 of the mouse and region 4 of the rat after the 15th day post-partum is statistically significant. The production of more epidermal cells might be expected on the lower surface of the foot, where greater friction is sustained by the skin surface (Thuringer, 1939) than on the trunk where the surface is protected by the hair coat; it is not clear, however, why more new cells are formed on the foot of the mouse than of the rat.

The difference between the average mitotic indices in the epidermis of the trunk of the mouse and the trunk of the rat after the 15th day post-partum is not statistically significant. For the adult animal, the surface area of which is



Text-fig. 18. The percentage numbers of four categories of dividing cells in the total population of nucleated epidermal cells. The ages are given in days after copulation or after birth. Thick broken lines: S. germinativum; thin broken lines: periderm; dotted lines: S. intermedium; thin continuous lines: S. spinosum.

almost constant, the duration of keratinization can be calculated from the mitotic index and the duration of mitosis (Glucksmann, 1945). Since in growing rats and mice the mitotic indices are not significantly different and the duration of mitosis is presumably the same, it might be assumed that keratinization proceeded at the same rate in both species. This would be true, provided the surface areas increased at the same rate, but in fact this is not so. During the first 100 days after birth the surface area of the rat increases more rapidly than that of the mouse (Lee, 1929; Grüneberg, 1943*b*). Some of the dividing cells in both species are used for lateral growth of the epidermis but more are used in the rat than in the mouse. It may therefore be concluded that keratinization takes longer in the rat than in the mouse. Glucksmann (1945) has shown that the time taken for keratinization in the adult mouse is approximately the same as the duration of the hair cycle (3–4 weeks). The hair cycle in the rat is longer, about 35 days (Butcher, 1934) and it is interesting that the duration of keratinization, also, is probably greater in the rat than in the mouse.

Cell division in the epidermis of the rat has been studied by Loeb & Haven (1929*a*), Carleton (1934, 1939) and Blumenfeld (1939) and in the epidermis of

the mouse by Picón (1933), Carleton (1934), Cooper & Franklin (1940), Blumenfeld (1943), Bullough (1943), and Cowdry & Thompson (1944). Picón has described a considerable decrease in mitotic frequency during the first month after birth, a slow rise to the 6th month and a slow fall to the 12th month after which the frequency remained constant. His results cannot be discussed in detail because the frequency has been expressed as the number of mitoses per unit surface area of epidermis and no allowance has been made for the decrease in epidermal height which occurs after birth. It is also unprofitable to discuss Carleton's results because she counted the dividing cells both in the epidermis and in the hair follicles, and the observations reported in the later paper were made after the injection of colchicine which arrests cell division at metaphase. Cooper & Franklin have investigated the diurnal mitotic rhythm in the epidermis of the ears of mice 15 days post-partum. The percentage of dividing nuclei in the total number of epidermal nuclei varied from 0 to 0·393. These values are considerably lower than the average (1·611) of my results for all four skin regions of a 15-day mouse; this disparity is presumably due to the difference in the sites that have been examined. Cowdry & Thompson found a mitotic frequency of 2·525 % in the epidermis of the hindfoot pads of mice 10 days after birth; in the region between the thenar and hypothenar pads, which is probably subjected to less friction than the pads themselves, I have found a frequency of 1·23 % in a mouse of the same age.

The determinations of mitotic frequency made by Blumenfeld (1939) on 28-day rats and by Blumenfeld (1943) and Bullough (1943) on adult mice cannot be discussed because they have been based on counts of dividing nuclei per unit volume (Blumenfeld) or unit area (Bullough) of epidermis; in addition Bullough injected colchicine before the tissues were fixed. Loeb & Haven (1929*a*) used rats of unstated age, presumably adult. The average percentages of dividing cells in the total epidermal cell population of the ears and chest were found to be 0·306 and 0·327 respectively. These values are lower than the average value (2·092) obtained for the four skin regions, trunk and foot, of a 100-day rat; the value for the chest epidermis is also lower than the value (2·13) for the anterior ventral region of this 100-day rat. Differences in age and site of epidermis may account for these divergences.

(3) *The height of the nucleated layers*

The height of the nucleated layers of the epidermis, i.e. the distance from the basement membrane to the outer limit of the S. granulosum has been measured at all stages of development; usually about ten measurements have been made at carefully selected positions in each specimen. The periderm has been excluded from the measurements. During part of embryonic development the outer layers of the epidermis are more folded than the inner layers, so that at the summits of the folds the epidermis is thicker than it is in the valleys of the folds (Pl. 1, fig. 3). In agreement with the observations of Fraser (1928) on the rat and Grüneberg (1943*a*) on the mouse these folds have been found to be most

conspicuous during the 19th day in the rat and the 17th day in the mouse. On the epidermis of these embryos equal numbers of height measurements at summits and valleys have been made and average values for epidermal thickness obtained. The epidermis of the three trunk regions of the youngest embryos is thinner medially than laterally; in these specimens the average height of the epidermis over the whole region has been found.

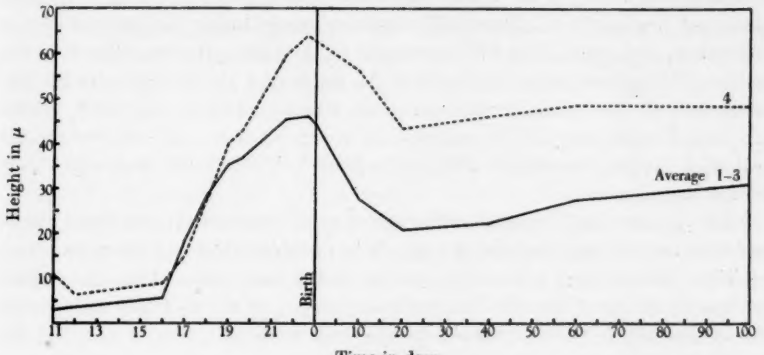
In Text-figs. 19 and 20 the height of the nucleated layers of the epidermis is plotted against the age of the specimen. The results for regions 1–3 of each animal are similar and have been averaged; the results for region 4 are given separately.

The changes in epidermal height are due almost entirely to changes in the number of strata and in the number of cell layers in each stratum (Table 2) but apparently are sometimes the result of changes in the height of individual cells.

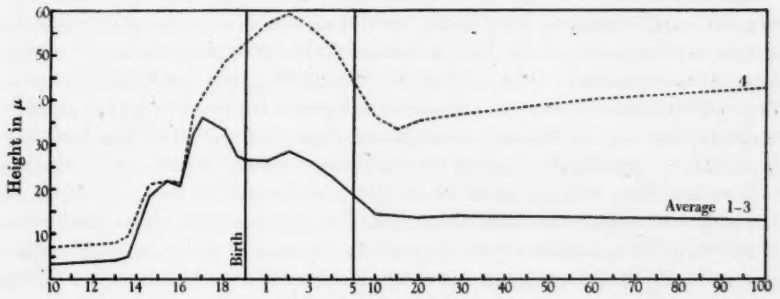
The decrease taking place in the height of the epidermis of the mouse just after birth is accompanied by a disorganization of the strata of nucleated cells indicating that the thinning of the epidermis may be due, at least in part, to lateral cell displacement during stretching; this suggestion has also been made by Gibbs (1941). She has found that from the 4th to the 13th days, correlated with the development of the tertiary and quaternary hair follicles, the epidermis on the anterior and posterior dorsal regions of the mouse 'decreased in actual thickness by a suppression of the cell layers of the strata granulosum and intermedium' (her 'stratum intermedium' corresponds to the S. spinosum of this paper). From the 13th to the 15th days the 'decrease in thickness of epidermis ceased, and a comparatively constant condition was maintained'. The graphs given by Gibbs for the anterior and posterior dorsal regions differ in two respects from Text-fig. 20: in the first place the epidermis was approximately 5μ thicker both before and after the decrease; and in the second place the main decrease did not begin until after the 4th day, whereas in Text-fig. 20 it is seen to begin after the 3rd day. Gibbs has found that the posterior dorsal epidermis is consistently thicker than the anterior dorsal epidermis between birth and the 15th day. A statistical treatment of my results and an examination of many more specimens would be necessary before reaching any conclusions about differences in epidermal height between the three trunk regions that have been studied; but, except in the 2-day animal, the epidermis from the posterior dorsal region of the mouse is apparently slightly thicker than that from the anterior dorsal region after birth.

The decrease in thickness of the post-natal epidermis of the mouse was noticed by David (1934) who found that it coincided with the completion of growth of the first pelage. The epidermis normally remained thin for the rest of life but could be made to regain its original thickness by various means: under the stimulus of grafting; after treatment with benzyl mercaptan; and after the application of collodion dressings. Hammet (1931) described the structure of the epidermis from the interscapular region of the adult mouse and its reaction to benzyl mercaptan but, as David pointed out, he did not realize

that the hyperplastic epidermis showed a close resemblance to the early postnatal epidermis. Picón (1933), who studied the frequency of mitosis in the epidermis of the mouse from birth to the age of 33 months, published a series of photographs of sections through the skin on the dorsum showing a striking decrease in epidermal thickness between the first and 7th days after birth, and a further decrease between the 2nd and 4th weeks. A decrease taking place



Text-fig. 19.



Text-fig. 20.

Text-figs. 19, 20. The height of the nucleated layers of the epidermis, excluding the periderm. Continuous lines: average of regions 1–3; broken lines: region 4. Fig. 19. Rat. 20. Mouse.

just after birth in the number of nucleated cells, both undifferentiated and differentiating, per unit surface area of interscapular mouse epidermis has been described by Glucksmann (1945). Fraser (1928) measured the height of each stratum of the dorsal epidermis during embryonic development in the rat; the aggregate heights of the S. germinativum (corresponding to the S. germinativum and S. spinosum of this paper) and the S. granulosum agree closely with the measurements that I have made.

DISCUSSION

(1) *Specific and regional differences*

Qualitative and quantitative studies on epidermal development have demonstrated differences between the mouse and the rat and between the lower surface of the foot (region 4) and the trunk, three parts of which have been examined (regions 1–3). These differences develop during the growth of the first pelage and reach their full expression at the time of its emergence on the surface. The epidermis on the trunk of the rat is then thicker than that of the mouse and all the strata are uninterrupted, whereas in the mouse the S. germinativum, S. spinosum and S. granulosum are incomplete. In both species the epidermis is thicker in region 4 than in regions 1–3 and in the mouse its strata are not disorganized.

These specific and regional differences can be correlated with the type of hair coat or with the absence of hair. It is probable that the closer and finer fur of the mouse gives a better protection to the skin surface than the coarser and less dense fur of the rat. On the lower surface of the foot hair follicles are absent and the friction sustained by the skin surface is greater than on the trunk. In other places where hair is absent in the mouse, e.g. around the anus and nipples and on the tail (Fekete, 1941) the epidermis is thick and stratified.

There are indications that these specific and regional differences in epidermal structure and thickness are related to differences in relative sensitivity to certain carcinogenic agents. It is well known that the epidermis of the mouse is much more sensitive than that of the rat (Glucksmann, 1945), and Twort & Twort (1936) have shown that the epidermis on the trunk of the mouse is more sensitive than the epidermis on the lower surface of the foot. Similarly the epidermis of genetically hairless rats has more cellular layers and a thicker S. corneum than that of their haired litter mates and is less susceptible to carcinogenesis induced by ultra-violet radiation (Hueper, 1941). The significance of the correlation between type of hair coat, epidermal structure and relative sensitivity to carcinogenic agents has been discussed by Glucksmann (1945) who concluded, however, that 'a specific sensitivity of the mouse epidermis to the growth-stimulating effect of the carcinogenic hydrocarbons must be assumed'.

Glucksmann (1945) has pointed out that there is no justification for the assertion, frequently encountered in the literature (David, 1934; Hammett, 1931), that the non-stratified epidermis of the adult mouse is 'undifferentiated' in comparison with the stratified 'differentiated' epidermis of the new-born mouse (David) or of the adult animal during wound repair or after treatment with chemical irritants (David; Hammett). He has shown that both undifferentiated (S. germinativum) cells and differentiating (S. spinosum and S. granulosum) cells can be identified in the thin nucleated part of the epidermis lying beneath the S. corneum on the trunk of the adult mouse; the observations

reported in this paper agree with his description. These observations indicate that cellular differentiation proceeds in a similar manner in both non-stratified and stratified epidermis. Further evidence is provided by the results of the analysis of the cell population (Text-figs. 14, 16), which show that the proportion of undifferentiated to differentiating cells is approximately the same in both species. Therefore, although stratification may be interpreted as an indication of *histological* differentiation, the modification or absence of stratification does not necessarily imply the modification or absence of *cellular* differentiation. Stratification is simply the result of the aggregation of cells into different layers according to the stages they have reached in differentiation.

(2) *Cell division and differentiation*

It has been found, with one exception, that in the rat and mouse the differentiating (keratinizing) cells of the epidermis do not divide and cell division is a function of the undifferentiated cells, viz. the cells of the S. germinativum and S. intermedium and those of the periderm before they begin to differentiate. There is one exception to this statement: for a short time after the formation of the S. spinosum during embryonic life its cells are able to divide. Division of spinous cells soon ceases on the three trunk regions but continues for a longer time on the lower surface of the foot. The cessation of division of spinous cells coincides with the time when the cytoplasm increases in amount and the intracellular fibrils become better developed. It may be suggested that the loss of viability is a result of the cytoplasmic changes involved in differentiation, such as the development of intracellular fibrils; or, as suggested by Dawson (1940), a single factor may both impair viability and initiate differentiation.

Although it has been widely accepted that cell division and cell differentiation are mutually exclusive (Lewis, 1939; Dawson, 1940) the division of cells located in the S. spinosum has been recorded several times. In the epidermis of the human scalp and prepuce Thuringer (1924, 1928) found not only that the cells of the S. spinosum were able to divide, but also that more than half of the total number of dividing cells in the whole epidermis were located in this stratum. In the epidermis of the pads on the feet of cats he recorded (1939) more dividing cells in the basal part of the S. spinosum than in either the basal layer of the epidermis or the more superficial parts of the S. spinosum. Cowdry & Thompson (1944) obtained similar results for the hind-foot pads of 10-day-old mice.

[They found that in normal animals the site of maximal mitotic frequency was the proximal third of the suprabasal part of the epidermis whereas after treatment with colchicine the site of maximal frequency moved to the middle third. They suggested that the level of maximal mitotic frequency 'is not fixed but subject to change in different physiological and pathological conditions'. In my opinion the results are to be interpreted as an expression of the normal upward migration of differentiating cells in the epidermis. Cells in which division has been arrested at metaphase by colchicine proceed to differentiate

and to migrate upwards through the epidermis thus increasing the apparent mitotic frequency of the more superficial parts of the epidermis.]

Cowdry, van Dyke & Geren (1946) have recently found that the site of maximal mitotic frequency is in the basal layer of the hyperplastic epidermis on the backs of young mice treated with methylcholanthrene. They attribute to the carcinogen the difference between this result and that of Cowdry & Thompson (1944).

As shown in the previous discussion cytological criteria are essential for judging the stage of differentiation reached by a cell, and the histological criterion of location in a particular stratum is of little significance without verification by cytological examination. Thuringer (1924, 1928, 1939) and Cowdry & Thompson (1944) and Cowdry *et al.* (1946) have identified cells by their position in the epidermis, a procedure which is apt to give misleading results when oblique sections, particularly in the neighbourhood of papillae or ridges, are examined. In such sections which, to judge from his photographs, were apparently used by Thuringer (1924), cells of the basal layers may appear to be situated more superficially. Moreover, Thuringer (1924) has recorded an average of 4 % of nuclei in the prophase of mitosis amongst all the dividing cells that he counted; but it is known from direct observations on dividing cells (Lewis, 1939) that prophase lasts for more than half the duration of mitosis. One must conclude that many of the dividing cells in early prophase were not recognized by Thuringer. In most epidermal cells, however, the early part of mitosis probably takes place while the cell is in the basal layer of the epidermis although some dividing cells may leave the basal layer before division is completed. The exclusion from his counts of these cells in prophase further invalidates his deduction that most of the dividing cells in the epidermis are spinous cells.

Other workers have found that nearly all the dividing cells in the epidermis of the mouse (Hammett, 1931; Picón, 1933) and of the rat and guinea pig (Loeb & Haven, 1929*a*, *b*) are situated in the basal layer. Two histological methods (Broders, 1920; Glucksmann, 1941) for judging the malignancy of human carcinomata are based largely on the belief, which has not been disproved, that a differentiating cell has lost the ability to divide and is no longer a dangerous source of new malignant cells.

GENERAL SUMMARY

1. A comparative account has been given of the histogenesis of the epidermis on the trunk and foot of albino mice and Norway rats aged 10 days post-coitum to 100 days post-partum.

2. Observations on cell division have led to the conclusion that in the epidermis of the mouse and rat differentiating cells do not normally divide.

3. Differences in epidermal development have been found between the rat and mouse and between the trunk and the lower surface of the foot. These differences can be correlated with the density of the hair coat or with its

absence. The type of epidermis which is relatively resistant to chemical carcinogenic agents (the epidermis of the rat and that of the mouse's foot) has been shown to differ from the type which is relatively sensitive (the epidermis of the trunk of the mouse).

This investigation was aided by emoluments from the Beilby Research Scholarship of Bedford College and the New Zealand Junior Research Scholarship of the British Federation of University Women, and by grants from the British Empire Cancer Campaign and the Sir Halley Stewart Trust. I am much indebted to Dr H. B. Fell for extending to me the facilities of the Strangeways Research Laboratory where the greater part of this investigation was made, and to Prof. H. Munro Fox, F.R.S., in whose Department at Bedford College it was completed. I wish to record my gratitude to Dr A. Glucksmann who suggested the work and who throughout its course gave his valued advice and encouragement.

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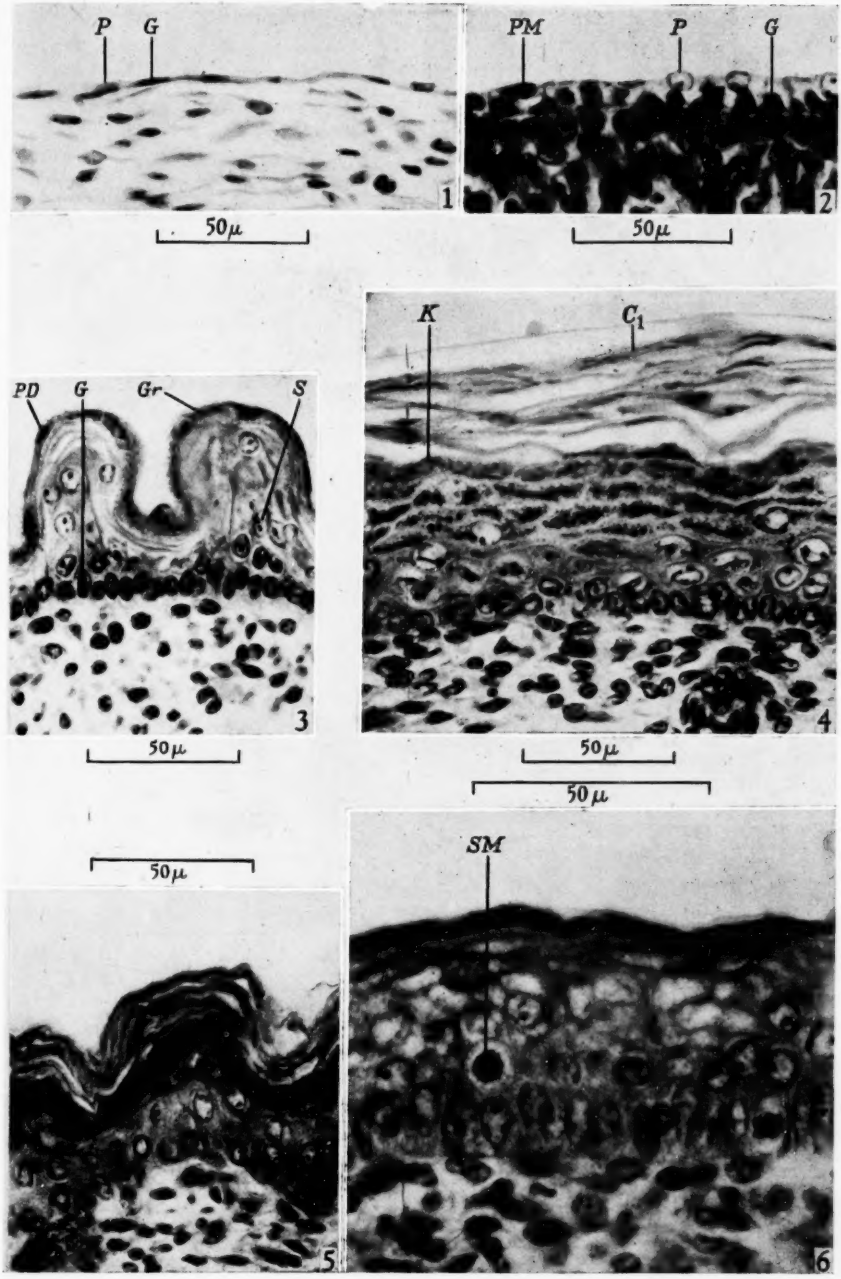
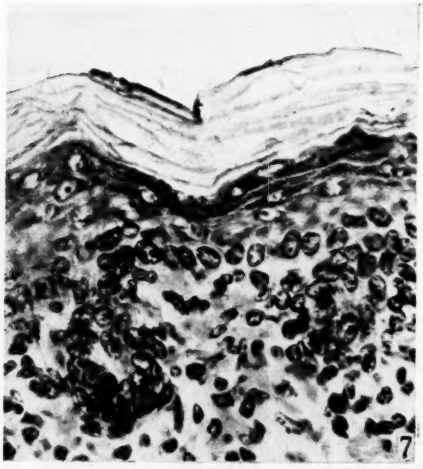


HANSON—THE HISTOGENESIS OF THE EPIDERMIS IN THE RAT AND MOUSE



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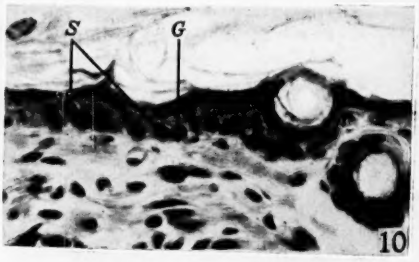

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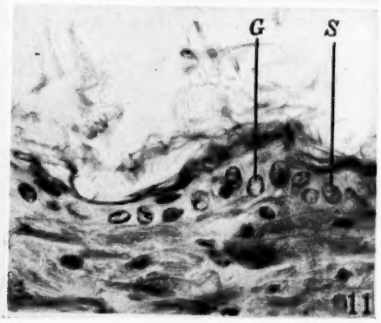

EXPLANATION OF PLATES

C_1, first-formed sheet of keratin; *E*, epitrichium; *G*, non-dividing cell of S. germinativum; *GM*, dividing cell of S. germinativum; *Gr*, S. granulosum; *I*, non-dividing cell of S. intermedium; *K*, large keratohyalin granule partly converted into eleidin; *L*, S. lucidum; *P*, non-dividing cell of periderm; *PD*, degenerating nucleus of periderm; *PM*, dividing cell of periderm; *S*, non-dividing cell of S. spinosum; *SM*, dividing cell of S. spinosum.

Fig. 1. The epidermis on region 1 of a 13-day mouse embryo. 8μ. Modified Mallory.
Fig. 2. The epidermis on region 4 of a 12-day rat embryo. 10μ. Modified Mallory.
Fig. 3. The epidermis on region 3 of a 19-day rat embryo. 8μ. Ehrlich's haematoxylin.
Fig. 4. The epidermis on region 2 of a 21-day rat embryo. 8μ. Ehrlich's haematoxylin.
Fig. 5. The epidermis on region 1 of an 18-day mouse embryo. 8μ. Modified Mallory.
Fig. 6. The epidermis on region 4 of a 16-day mouse embryo. 8μ. Modified Mallory.
Fig. 7. The epidermis on region 2 of a mouse one day after birth. 8μ. Ehrlich's haematoxylin.
Fig. 8. The epidermis on region 4 of a mouse 4 days after birth. 8μ. Modified Mallory.
Fig. 9. The epidermis on region 2 of a rat 10 days after birth. 8μ. Ehrlich's haematoxylin.
Fig. 10. The epidermis on region 1 of a mouse 10 days after birth. 8μ. Ehrlich's haematoxylin.
Fig. 11. The epidermis on region 2 of a mouse 60 days after birth. 10μ. Ehrlich's haematoxylin.

IN MEMORIAM

REGINALD JOHN GLADSTONE, M.D., F.R.C.S., F.R.S. Edin.

It is with great regret that we record the death on 12 February 1947 of Dr R. J. Gladstone, till 1938 Reader in Anatomy and Lecturer in Embryology at King's College, London.

Dr Gladstone was born in 1865, the son of Dr T. H. Gladstone, and was educated at Clapham Grammar School. He studied medicine in Aberdeen and obtained the M.B. degree of that university in 1888, the year after the foundation of the Anatomical Society, also the degree of Master of Surgery, and the D.P.H. In 1894 he became M.D. Leaving Aberdeen, he was appointed House Physician and later House Surgeon at the Middlesex Hospital. He then decided to turn to Anatomy, and he was appointed a Junior Demonstrator in the Middlesex Hospital Medical School in 1895, and Senior Demonstrator the next year. It was in this year also that he obtained the Diploma F.R.C.S., England. In 1905 he went to Vienna with Dr R. A. Young and studied the methods of teaching Anatomy and Surgery. In 1906 he visited Vancouver as a representative of the British Medical Association and returned with further new ideas on the education of medical students. He remained on the staff of the Middlesex Hospital Medical School till 1913. In that year he was appointed Reader in Anatomy at King's College and Lecturer in Embryology, the posts which he held at his retirement in 1938.

Gladstone was one of the oldest members of the Anatomical Society, being elected a member in 1897, the year in which Waldeyer, His and Le Boucq were elected the first honorary members of the Society. It was in this year that his first paper in the *Journal of Anatomy*, vol. XXXI, was published, on 'A case of an additional presacral vertebra'. From this time onwards he continued to make valuable contributions to the *Journal*, his last scientific paper, on 'A presomite human embryo (Shaw)' in collaboration with W. J. Hamilton, appearing in two parts in vol. CXXVI, 1941 and 1942.

Gladstone often collaborated with others, and he and C. P. G. Wakeley published several joint papers. In 1940 they produced a scholarly monograph on the pineal organ, in which work Gladstone's profound knowledge of comparative embryology was seen. One of his most important papers in the *Journal of Anatomy* appeared in 1932, 'On the morphology of the sternum and its relation to the ribs'. He was an authority also on that difficult subject, the development of the veins of the dorsal body wall.

Many of Gladstone's papers dealt with abnormalities, from which he was always able to draw an interesting and stimulating lesson and his accounts were much more than a mere record. He had considerable artistic ability, and himself drew the diagrams that adorned many of his papers.

Gladstone was not only a regular attendant at the Meetings of the Anatomical Society but served it faithfully and well for many years as Recorder. There was a break from 1909 to 1925 in the publication of the *Proceedings* of the Society, and Gladstone was the first to occupy the regular office of Recorder of the Society. He recorded, and Barclay Smith edited, the *Proceedings* of the Society from 1925 to 1936, when he was succeeded by A. J. E. Cave. In this connexion it is interesting to note that Gladstone's last publication in the *Journal* was the 'In Memoriam' notice of his old friend and chief, Prof. Barclay Smith, who died on 5 July, 1945.

It was as an embryologist that Gladstone was best known. He had, indeed, as wide a knowledge of human and comparative embryology as any one. Gladstone was the kindest and humblest of men, and he was loved by all. Though he was very shy he always had a kindly word and friendly greeting for all those he knew, young and old alike. In spite of his wide knowledge he was most self-effacing, indeed he was one of those who should 'inherit the earth'. We shall all miss his smile behind his strong glasses and his shy welcome to Meetings of the Society. All those who knew him will sympathize with his wife and family in the great sorrow that must be theirs.

We are greatly indebted to Gladstone's old friend, Sir Cecil Wakeley, for many interesting details of his career.

NOTICES OF BOOKS RECEIVED

Researches on Pre-natal Life. By Sir JOSEPH BARCROFT. (Oxford: Blackwell Scientific Publications.) Vol. I, pp. xiii + 292; 124 figs.; cr. 4to; 37*s.* 6*d.* net.

As the author explains in an entertaining Preface the book contains such information as he has collected concerning pre-natal life. It deals with his own work, or at least work in which he has had a hand, and not to any great extent with work with which he has had no personal contact. This, the first of two volumes, deals particularly with vascular and respiratory problems—placental barrier, cardiac output, vascular reflexes, carotid blood, closure of foramen ovale. The sheep is the animal dealt with chiefly. A full bibliography appears at the end of each chapter. A thought-provoking and stimulating work.

A Prelude to Modern Science (The *Tabulae Anatomicae Sex* of Vesalius). By CHARLES SINGER and C. RABIN. (Cambridge University Press, for the Wellcome Historical Medical Museum.) Pp. lxxxvi + 58; 59 text-figs. and 6 Plates; med. 4to; 50*s.* net.

The first 86 pages contain 'a discussion of the History, Sources and Circumstances' of Vesalius's six anatomical Plates of 1538. An account is given of Vesalius's early life and work at Paris and Louvain, of his Italian predecessors, of the evidence of animal sources for the *Tabulae* and of the influence of Renaissance and Semitic vocabularies on the nomenclature employed. A facsimile of each of the six Tables is bound into the end of the book, and the latter part of the work contains a translation into English of the text which adorns and surrounds each Plate. The translation is richly annotated. There is a General, Greek, Arabic and Hebrew index. The whole work is an exhaustive and scholarly discussion from every point of view of the *Tabulae Anatomicae Sex.*

Human Genetics. By R. RUGGLES GATES. 2 vols., pp. xvi + 742—1518, 42 tables, 326 figs., including pedigrees. Med. 8vo. £5 net. London and New York: The Macmillan Company.

An essentially biological approach to genetics of man, 'intended as a source book for all those concerned with medicine or anthropology in its genetic aspects'. A comprehensive, fully documented, well-indexed, well-bound and printed contribution to the study of genetics, dealing with almost every imaginable trait, habit or disease that man is heir to.